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THE UNIVERSITY OF ALBERTA

A STUDY OF GENE ACTION AND STABILITY IN ALFALFA CLONES PREVIOUSLY SELECTED FOR RESISTANCE TO ALFALFA SICKNESS

by

FELICITAS MPUNDU KATEPA

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF MASTER OF SCIENCE

IN

PLANT BREEDING

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled A STUDY OF GENE ACTION AND STABILITY IN ALFALFA CLONES PREVIOUSLY SELECTED FOR RESISTANCE TO ALFALFA SICKNESS submitted by FELICITAS MPUNDU KATEPA in partial fulfilment of the requirements for the degree of MASTER OF SCIENCE in PLANT BREEDING.

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ABSTRACT

Three diallels two in the field and one in the glasshouse, were employed to study the gene action and stability of dry matter yield in eight alfalfa clones which were previously selected for their resistance to alfalfa sickness. From 1982 to 1984, nine forage harvests were made from the two field diallel crosses and these represented the different environments for which the Eberhart-Russel stability analysis of the clones was employed. A fourth experiment, in the glasshouse, was conducted to compare the performance of resistant and susceptible strains in sterilised and unsterilised sick soil (a soil which produces alfalfa sickness). The glasshouse tests were evaluated for plant height, leaf weight, leaf area, total dry weight, specific leaf weight and root necrosis.

Griffing's analysis of the field diallels indicated that additive gene action was important in determining dry matter yield among the alfalfa clones. The clones were evaluated for dry matter yield, general combining ability (G.C.A.) and stability with a view to selecting suitable clones for inclusion in a synthetic strain. All clones were stable with mean square deviations not significantly different from zero. Except for the clone 1VP9, the clones had a significant linear relationship with the environment. A significant regression was obtained for 1VP9 when log transformed mean yields were used.

On the basis of this study it was recommended that clones 2G169, 2B76 and 1VP9 with high dry matter yield and G.C.A., constitute parental clones for a synthetic strain. Clone 2B29 with average dry matter yield and G.C.A. should be included as a fourth parent to ensure the maintenance of a broad genetic base and prevent inbreeding depression in the advanced generation of the synthetic.

The glasshouse diallel, grown in alfalfa sick soil indicated that previous selection had depleted additive genes and success in further selection for decreased root necrosis could not be expected. There is a need to identify the pathogen(s) and environment(s)s which cause and enhance alfalfa sickness so that these may be employed to increase the selection pressure in the breeding programme.

Strains previously selected for their resistance to alfalfa sickness had larger leaf areas and higher leaf weights than those selected as susceptible strains. Resistant strains could therefore be expected to exhibit superior net assimilation rates.

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I. INTRODUCTION

Alfalfa, the queen of forage crops, is the most widely used and the oldest forage in the world. It originates from Iran, Transcausia and Asia Minor, and historical records indicate that it was a valuable crop in Persia and Turkey as early as 1000 B.C. Alfalfa was introduced to Greece during the Persian Invasion of 490 B.C. from where it spread to Rome and subsequently throughout the Roman empire. The discovery of the New World, Australia, and New Zealand, in the 18th century and the colonization of the Americas in the 16th century resulted in the worldwide distribution of alfalfa. From Peru, the crop spread to Chile, Argentina and Uruguay, and it was introduced to the southern United States of America from Mexico. In 1871 the first Canadian introduction of alfalfa was made in Welland, Ontario from France. It lacked climatic adaptation for western Canada but winterhardy cultivars, Grim and Baltic were introduced to western Canada from the United States of America.

Alfalfa is grown in temperate and subtropical climates on over 30 million hectares. Appendix 1 gives an estimate of the world distribution of alfalfa. Canada, the USA and Mexico produce 40 percent of the crop with Europe, South America, Asia, Africa and Oceania producing 28, 24, 4, 1 and 4 percent respectively. There has been a significant increase in production from 1962 when Bolton reported a total area of approximately 20 million hectares. The wide adaptation and success of alfalfa may be attributed to the

existence and maintenance of great genetic variability by both the diploid (2n=16) and tetraploid (2n=32) forms through cross pollination. The deep root system coupled with its symbiotic relationship with *Rhizobia* contribute significantly to the crop's success. The creeping roots, rhizomes and deepset crowns provide protection against heaving and severe cold winters. Alfalfa can remain dormant under stress and resumes growth when favourable conditions prevail.

A. THE PLANT

Alfalfa exhibits great morphological and physiological diversity as a result of its different ploidy levels, its wide geographical distribution and cross pollination. Common alfalfa (*Medicago sativa*) to which the varieties Alfa, Du Puits and Glacier belong, is purple flowered, high yielding and susceptible to winter injury, while the winterhardy yellow flowered *M. falcata* is of minor agricultural importance. Many commercial cultivars such as Roamer, Rambler and Vernal are hybrids of *M. sativa* and *M. falcata* and are commonly called *M. media* or variagated alfalfa.

Alfalfa is a medium-lived perennial plant, one to three metres tall with a deep root system which penetrates three to six metres into the ground. *M. sativa* is tap rooted with a narrow crown, and an upright branched stem bearing wide trifoliate compound leaves. *M. falcata* has a branched root system with fine stems and lanceolate leaves. The leaves are

serrated towards their apices and bear a prominent midvein with pinnately branched lateral veins. There are slender stipules at the base of the petioles. The inflorescence is a ten to forty flowered oblong to spherical raceme and pod shapes vary from sickle, characteristic of *M. falcata*, to the three and four coils of *M. sativa*. The sickle pods have been selected out of most commercial cultivars due to their relatively low seed yields. Variability has also been observed in photosynthetic capacity within and between cultivars. (Barnes *et al.*, 1969,).

Alfalfa is adapted to a wide range of climatic and soil conditions but grows best on soils with a high lime content or near neutral soils. This crop has good salt and drought tolerance but is intolerant of acidity, high alkalinity and waterlogging. In Alberta, alfalfa is the preferred hay crop because of its high forage yield with a good second cut. It is valuable for its outstanding palatability and high protein content of about 24 to 26 percent in the leaves and 8 to 10 percent in the stem at early flowering. (Ag. Canada Publication 1981). Alfalfa provides an important source of inexpensive plant protein. Its use as a pure pasture stand is limited by its high bloating potential, however pure alfalfa stands are common among producers of seed and dehydrated products.

Alfalfa seed, dehydrated products and tame hay in pure and mixed stands together represent a multi-million dollar industry. Approximately 56 percent of the tame hay in Canada

contain alfalfa. In Alberta an estimate of 5,805,950 tonnes of tame hay valued at 423,299,000 dollars was produced annually over the last three years. Western Canadian dehydrated alfalfa products are valued at 37 million dollars annually, representing 300,000 tonnes of production from 59,000 hectares of intensively managed alfalfa. Alberta produces 41 percent of this and together with Saskatchewan make up 88 percent of the total production. Certified seed production is limited mostly to the Prairie Provinces of Manitoba, Saskatchewan and Alberta (Appendix 2) with an average total of approximately 2 tonnes per year, on over 119,000 hectares. Of this, Alberta produces 34 percent.

B. THE DISEASE

The term alfalfa sickness refers to a specific condition that causes poor growth of alfalfa in north and central Alberta (Webster *et al.*, 1967). It was first observed in 1962 (Goettel) on light textured soils. Alfalfa sickness is characterised by stunted and spindly growth of young plants with yellowish leaves which become flaccid and bear irregular necrotic patches. There are brown lesions on the roots, particularly the lateral roots. Nodulation is inhibited and the roots become girdled leading to the collapse of the entire plant. The nodules are either absent or appear in large whitish clumps. The sickness has been observed only in fields which previously contained alfalfa and there are characteristic irregular patches of healthy

growth amongst the poor growth. Comparative photographs taken by Webster *et al.* (1967), Damirji *et al.* (1976, 1978) show the above characteristics. Differences in plant height and rooting between healthy and sick plants are marked.

Questions pertaining to the causal factors of alfalfa sickness have not been resolved. Investigative studies by Webster *et al.* (1967) determined that the sickness is not caused by a deficiency or excess of soil micro and macro mineral nutrients, and soil moisture. Furthermore, the sickness is not related to nematodes (Webster and Hawn 1973; Damirji *et al.*, 1976). Soil sterilization eliminates the sickness leading to the conclusion that the primary cause of alfalfa sickness is biological in nature and various fungi including *Phytophthora megasperma*, *Fusarium*, *Pythium*, and *Cylindrocarpon* species have been implicated. Upon testing *P. megasperma* produced disease symptoms very similar to alfalfa sickness but it has not been consistently isolated from lesions on roots of sick plants. Furthermore, *P. megasperma* infection is known to be most prevalent in heavy, poorly drained soils, a category into which the alfalfa sick soils of Alberta do not fit. Thus there is need to establish the causal factor(s) of alfalfa sickness.

C. ECONOMIC IMPORTANCE

No survey has been conducted to establish the spread, severity and economic significance of Alfalfa sickness but Webster *et al.* (1967) indicates that it is widespread in

central Alberta. Alfalfa sickness reduces productivity and stand longevity. Establishment costs in the seeding year are conservatively estimated at 300 to 370 dollars per hectare with little or no return because of slow initial growth (Ag. Canada publication, 1982). Longevity and high productivity are critical from an economic standpoint.

D. SELECTION

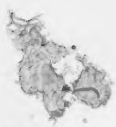
Initial efforts to breed for reduced alfalfa sickness were unsuccessful (Goplen and Webster 1969), however Faechner and Bolton (1978) demonstrated increased resistance to the sickness as a result of selection. After three cycles of recurrent selection they obtained a 14 percent reduction in root necrosis, a 39 percent increase in plant height which contributed to an 80 percent increase in dry matter yield. Selection was based on plant phenotypic traits *i.e.* height and root necrosis, and it culminated in the development of six synthetic strains four of which are being tested in seed and dry matter yield trials.

Present research aims at improving the selection criteria and crossing strategy in the breeding programme, and at determining the genetics of plant reaction to alfalfa sickness. The study has three major objectives:

1. to improve the breeding strategy and selection criteria of the breeding programme,
2. to determine some genetic parameters of plant reaction to alfalfa sickness in alfalfa clones selected for

resistance to alfalfa sickness,

3. to evaluate the stability in yield of material which has been selected for relatively low root necrosis and high dry matter yield in alfalfa sick soil.



II. LITERATURE REVIEW

Alfalfa sickness was first observed by Goettel in 1962 on light textured dark grey luvisolic soils, with a pH range of 5.6 to 6.7. Webster *et al.* (1967) considered that the sickness might be attributed to toxic substances in the soil, which inhibited healthy growth of subsequent alfalfa crops. A similar pattern of poor growth has been reported in Idaho, where liming eliminated the sickness (Harder *et al.*, 1962) and in Washington (Weber and Leggett, 1966) where it was attributed to ineffective rhizobia. The sickness in Canada has not been observed in other crops such as red clover and birdsfoot-trefoil (Webster *et al.*, 1967).

Field and glasshouse studies conducted in 1962 and 1965 in central Alberta indicated that alfalfa sickness was not caused by a deficiency or an excess of soil moisture(Webster *et al.* , 1967). The rainfall was above average and sick soil profiles revealed moisture to a depth of 150 cm. Low subsoil moisture might be eliminated as the cause of alfalfa sickness since a watertable was found at depths of 270-330 cm in areas of good growth. Applications of nitrogen, phosphorus, potassium, sulphur, lime and micronutrients did not prevent the development of the sickness (Goplen and Webster, 1969; Webster *et al.*, 1967) so that nutrients were not a factor. Alfalfa sickness was effectively controlled by heat and chemical sterilization of the soil (Faechner and Bolton, 1978; Webster *et al.*, 1967). Vapam (4-5(CH_3-NH.CS.S.Na.$2H_2O$), which is a temporary soil

sterilant eliminated all symptoms of the sickness. Thus the primary cause of alfalfa sickness was believed to be biological in nature but has not been identified.

Webster *et al.* (1972) studied the relationship of the nematode *Paratylenchus projectus* to alfalfa sickness on the recommendation of W.R. Orchard who had from 1962 to 1969 consistently found high counts of the nematode in alfalfa sick soils. This finding was verified in a preliminary survey of north and central Alberta (Webster *et al.*, 1972). A more extensive survey (Webster and Hawn, 1973) revealed that counts of *P. projectus* were neither related to soil parameters nor to the cropping history of an area. Inoculation of alfalfa seedling with *P. projectus* (Damirgi *et al.*, 1976) did not produce alfalfa sickness symptoms. The high counts of *P. projectus* were thus attributed to the presence of decayed roots from alfalfa sick plants.

Pathogenicity studies of root lesions on alfalfa sick plants identified *P. megasperma* as a primary causal agent of the sickness (Damirgi *et al.*, 1978, 1979). Several fungi (*Pythium* sp, *Altenaria* sp, *Fusarium* sp, *Trichodermas* sp, *Phytophthora* sp) and bacteria were isolated but only *P. megasperma* produced disease symptoms similar to alfalfa sickness in seedlings. An extract from sick soil produced alfalfa sickness, and seedlings displayed a common reaction to alfalfa sickness and *P. megasperma* infection.

A. PHYTOPHTHORA MEGASPERMA

P. megasperma root rot is characterised by the destruction of most fine lateral roots with large taproot lesions originating from spongy phellem cells at the base of the lateral roots. There are restricted taproot lesions associated with the wound periderm formed around the infected area (Bushong and Gerderman, 1959; Erwin, 1954; Marks and Mitchell, 1970). This causes foliar discolouration, stunting and death of the plants. These symptoms are similar to alfalfa sickness.

P. megasperma root rot has a wide geographic distribution in North America and Australia. It has been reported in California (Erwin, 1954), Illinois (Bushong and Gerderman, 1959), Arizona (Hine *et al.*, 1972), Ontario (Chi, 1966), Wyoming (Gray *et al.*, 1983), New York (Wilkinson and Millar, 1982), Australia (Bray and Irwin, 1978; Purss, 1969) and Mexico (Aguirre *et al.*, 1983). The distribution of *P. megasperma* varies within a soil profile. Gray and Hine (1976) recovered the fungus at depths up to 56 and 80 cm in a clay and a loam soil respectively. Most root lesions were observed from 4 to 25 cm below the soil surface. Erwin (1954) first reported *Phytophthora cryptogea* as the cause of this root rot but later (1965) reclassified the pathogen as a specialised strain of *P. megasperma* Drechs. The fungus is associated with poorly drained or heavily irrigated soils and excessive rainfall (Pratt and Mitchell, 1973), while alfalfa sickness is not associated with these conditions

(Webster *et al.*, 1967). *P. megasperma* and alfalfa sickness both reduce yield most drastically in juvenile plants (Purss, 1969; Webster *et al.*, 1967; 1972). Gray and Hine (1976) observed that the pathogen initially infects seedlings at 4 to 8 weeks of age, similar to the initial infection time for alfalfa sickness (Faechner, 1977). These diseases are both very persistent in the soil, consistent with the long survival of oospores and chlamydospores of *P. megasperma* (Pratt and Mitchell, 1975). *P. megasperma* remained infective after 3 to 9 months of storage at 4°C at high moisture levels. At moisture levels below 40 bar tension, pathogenicity was greatly reduced. Susceptible cultivars in a naturally infected soil increased the activity of the fungus more than resistant cultivars, and the activity declined in the presence of non-host crops such as maize, oats, clover, soybean and peas. *P. megasperma* is therefore a poor saprophyte. Ham and Hansen (1981) reported a strong host specificity of *P. megasperma* for alfalfa, however, Wilcox and Miretich (1982) demonstrated a host non specificity of *P. megasperma* isolates recovered from fourteen different plant species.

P. megasperma sporulates in cool (15-20°C) moist conditions, with moisture being the most important determinant followed by temperature (Frosheiser, 1969; Johnson and Morgan, 1965; Pratt and Mitchell, 1973, 1975). Spore germination and hyphal development is affected by the chemical composition of the microflora. Levels of *P.*

megasperma inoculum increased upon the introduction of alfalfa seeds or seedlings to the soil in both glasshouse and field studies (Pratt and Mitchell, 1973). Zoospore attraction to alfalfa roots was demonstrated by Marks and Mitchell (1970).

B. INFECTION

Zoospores are the principle propagule responsible for seedling infection by *P. megasperma* (Wilkinson and Millar, 1982), however Basu (1981) reported the existence of chlamydospores as a soil survival and primary infective propagule. Initial root infection was associated with root nodules (Gray and Hine, 1976), the region of cell division and cell extension at the root apex, and the junction of the lateral and tap roots (Irwin, 1976; Marks and Mitchell, 1971). There was a 24 percent increase in seedling death upon infection with *Rhizobium meliloti*. Zoospores mostly encyst at these infection sites where they germinate and penetrate the root with an infection peg. Marks and Mitchell (1971) reported that no appressoria were formed, and hyphal growth within the root is both inter and intracellular causing extensive damage to the vascular systems in young material while being mostly restricted to the cortex in older plants. Consequently root rot damage is minimal in lignified tissue but results in rapid death of young plants. Post-emergence damping off has been reported by Bushong and Gerderman (1959), Smittenhenner (1964), Johnson and Morgan

(1965) and Gray *et al.* (1973) while pre-emergence damping off has been reported by some workers (Bushong and Gerderman, 1959; Gray *et al.*, 1973) and disputed by others Johnson and Morgan, 1965; Smitthenner, 1964). The literature does not report seedling damping off in association with alfalfa sickness. The temperature range favouring infection is wide (17-27 °C), the optimum being 20-24°C (Erwin, 1965; Pratt and Mitchell, 1975). Wilkinson and Miller (1982) reported that root rot was directly proportional and plant survival inversely prportional to soil moisture. This was attributed to a possible increase in available nutrients from the roots due to leakage and diffusion in the high water medium (Ta-Li Kuan and Erwin 1982).

The evidence for *P. megasperma* being the primary cause of alfalfa sickness is strong but inconclusive. *P. megasperma* is known to be most prevalent in heavy poorly drained soils and in areas of frequent heavy rainfall or irrigation. This is not consistent with alfalfa sickness, and *P. megasperma* is not consistently isolated from root lesions of alfalfa sick plants. Reeleder (1982) concluded that *P. megasperma* does not cause alfalfa sickness but other organisms including *Cylindrocarpon gracile* and *Fusarium roseum* do. Reeleder also concluded that low soil fertility (particularly sulphur) contributes to alfalfa sickness. Inoculating alfalfa seedlings with *P. megasperma* produced symptoms which were different from alfalfa sickness, a finding which contradicts work done by Damirgi (1978) and

differs from the description of *P. megasperma* root rot by Marks and Mitchell (1970), Erwin (1954) and Bushong and Gerderman (1959). Furthermore, soil nutrient status was earlier discarded as a major factor in alfalfa sickness (Webster *et al.*, 1967). It is important to note that the survey by Reeleder (1982) was conducted mostly on soils which are inherently low in mineral nutrients particularly sulphur and this could be the reason for Reeleder's association of the sickness with low soil nutrients. Alfalfa sickness primarily affects juvenile plants but Reeleder worked with randomly chosen established fields. It is therefore possible that Reeleder was studying alfalfa root rots different from alfalfa sickness. Further controversy was presented by Hawn and Kozub (1978) who identified *P. projectus* as the cause of alfalfa sickness in combination with low soil pH, and low soil fertility. Stelfox and Williams (1980) identified Pythium species as a cause of alfalfa sickness.

The reasons for these contradictory findings are not clear. The age of the plants under study varies among the workers. Webster *et al.* (1967), Damirgi *et al.* (1976, 1978, 1979), and Faechner and Bolton (1978a and b) worked with juvenile plants while Reeleder (1982), Hawn and Kozub (1978), and Stelfox and Williams (1980) surveyed in a range of plant ages. The sick soil as observed by Goettel (1962) and Webster *et al.* (1967) had a light textured surface horizon while Reeleder sampled heavy soils. The definition

of alfalfa sickness is ambiguous because the causal factors are unknown. The sickness is thus open to numerous possibly contradictory interpretations depending on plant age, soil types, moisture and nutrient status and the predominant organisms. For the purpose of this study, alfalfa sickness is defined as poor growth of juvenile alfalfa bearing the disease symptoms described on page 4 Chapter I. B from Webster *et al.* (1967). This definition has been used by Damirgi *et al.* (1976, 1978, 1979), and Faechner and Bolton (1978a and b).

C. BREEDING

> "Breeding for resistance to disease has been defined as selection for the inherent capacity of a plant to prevent or restrict entry or subsequent activities of a pathogenic agent when the plant is exposed, under suitable environmental conditions to sufficient inoculum of a pathogen to cause disease."

Kerh *et al.*, 1972.

Medicago sativa is a cross pollinated autotetraploid and exhibits great genetic variability. Selection has been conducted for numerous traits including productivity, growth type, chemical and structural composition, persistence in various environments and management regimes, seedling vigour, recovery after forage harvest, dormancy and resistance to many diseases, nematodes and insect pests. Various methods and combinations of introduction, selection,

crossing and evaluation have been employed. Selection for resistance to alfalfa sickness is complicated by the undefined nature of the pathogen, and host-pathogen interaction. Recurrent phenotypic selection is an effective method of breeding for alfalfa disease resistance and has been utilised in the development of resistance to rust (Hill *et al.*, 1963), common leaf spot (Graham *et al.*, 1965), bacterial wilt (Barnes *et al.*, 1971), anthracnose (Devine and McMurtrey, 1975) and *P. megasperma* root rot (Hine *et al.*, 1975). The success in using recurrent phenotypic selection is attributed to the increased frequency of favourable genes by repeated recombination resulting in the development of new genotypes, and consequently new phenotypes.

Inheritance of resistance to most diseases of alfalfa is controlled by a single tetrasomic locus sometimes with incomplete dominance (Goplen and Stanford, 1960; Kehr *et al.*, 1972; Lu *et al.*, 1973; Pederson and Barnes, 1965). Lu *et al.* (1973) found that susceptibility in alfalfa to *P. megasperma* infection was conditioned by a single incomplete dominant tetrasomic gene and Irwin *et al.* (1981) observed that the segregation for disease reaction in the S_1 and F_1 populations suggested resistance to be conditioned by two incompletely dominant genes. An arrangement of at least a duplex genotype at one locus and a simplex at the other locus was required before resistance was expressed. Thus there may be two or more genetic systems controlling alfalfa

reaction to *P. megasperma*. This is feasible because of the great genetic variability existing in alfalfa. Elgin (1979) suggests that resistance to the stem nematode in alfalfa is conferred by at least a simplex at each of two complementary loci.

Variability in alfalfa reaction to *P. megasperma* has been identified. Erwin (1966) showed alfalfa cultivars Arabia #65 and #64 to be more resistant than Lahanton which was in turn more resistant than California common 49, Hilma and Africa. On a scale of 1 to 6 Frosheiser and Barnes (1973) rated six cultivars of alfalfa as follows: Agate 2.7, Lahanton 3.0, Rambler 4.2, Ladak 4.2, Vernal 4.6 and Grimm 4.8 in decreasing order of resistance. Complete resistance of alfalfa to *P. megasperma* is unknown but several cultivars and strains have been released as resistant material in the United States of America. These include Agate (Frosheiser and Barnes, 1973), CU 38 and UC 47 (Lehman *et al.*, 1969) and Rincon (Melton *et al.*, 1979). In Mexico, Aguirre *et al.* (1983) have developed a resistant cultivar.

The morphological and physiological basis of plant resistance to *P. megasperma* has not been identified. Marks and Mitchell (1970) associated resistance to larger diameter central steles of the roots, increased lateral rooting and a hypersensitive type of cortical cell reaction, the nature of which is unknown. The rejection of the pathogen by the plant is seen at the cortical cell level and the endodermis in resistant cultivars. Susceptible and resistant host plants

cannot be distinguished by response time or massing of *P. megasperma* zoospores to the roots since the observed chemotaxis is unselective (Dukes and Apple, 1961; Irwin, 1976; Marks and Mitchell, 1970). Thus resistance is conferred after penetration of the host by the zoospore germ tube.

Marks and Mitchell (1971) and Erwin (1966) reported that resistance to *P. megasperma* was not expressed at the seedling stage but Gray *et al.* (1973) observed enhanced resistance in the progeny of plants which survived pre and post emergence damping off. Damping off could be a useful trait in breeding against *P. megasperma* root rot. Pratt *et al.* (1975) observed that plant reaction to the fungus was expressed in cotyledons of 3 to 10 day old seedlings. The cotyledons of susceptible plants shrivelled after inoculation with zoospores while those of resistant plants merely developed red brown localised necrotic lesions on the upper surfaces. Cotyledon reaction was significantly correlated with root rot.

There is a source of resistance to *P. megasperma* within the cultivated alfalfa and closely related diploid and tetraploid *Medicago* species (Irwin *et al.*, 1981) which may be used to develop more resistant cultivars using the current phenotypic selection in both seedling and adult plants.

Control of alfalfa sickness by chemical, physical and plant breeding means has been studied by Faechner and Bolton

(1978a, 1978b). Cultivars of *Medicago media* namely Beaver (B), Vernal (V), Grimm (G), and Roamer (R) were evaluated over three cycles of recurrent selection in growth room and field experiments. Phenotypic selection was based on plant height, yield and root necrosis. General and specific combining abilities of progenies from a) six resistant and b) five susceptible selected parents were analysed. Also evaluated were the effects on alfalfa sickness of four fungicides (Benlate (benomyl), Dexon (Fenameno sulphur), Metazoloxan (drazoloxan) and Dowco 269 (nurelle), steam and chemical (Vapam) sterilization and soil pH on alfalfa sickness. The final test was a comparison of *P. megasperma* infection to alfalfa sickness.

Results confirmed the similarity of alfalfa sickness to *P. megasperma* infection, and demonstrated the effectiveness of soil sterilization, chemical treatment (Dowco 269) and plant breeding. Of these control measures, plant breeding is the least expensive and most practical. Disease was negatively correlated to plant height and dry matter yield and after one selection cycle disease rating decreased by 10 percent while height and dry matter yield increased by 11 and 18 percent respectively. After three selection cycles, the corresponding percentages were 14, 39 and 80 percent. The predominance of the general combining ability effects suggests a large additive genetic variation.

From these studies the lines 2V96, 2R163, 2R187, 1B29, 1B73, 2B76, and 2V13 were recommended for inclusion in a

disease resistant synthetic while 1GP20, 1GP21, 1GP130, 1R11, 1R118 and 1VP19 were suitable for synthesising a susceptible strain. The number before the cultivar name designates the cycle of selction while the number following the cultivar was arbitrarily given in 0 selection cycle. These clones were recommended on the basis of dry matter yield, plant height, root disease rating and general combining ability.

Faechner's recurrent selection culminated in the development of six resistant synthetic strains four of which are undergoing yield tests in western Canada.

D. DIALLELS

A diallel cross is a mating design in which individuals of a group are mated with each other in all possible combinations. Diallels have been employed to evaluate parental lines on the basis of the performance of their progenies (with or without the parental lines) and have been used for the analysis of components of genetic variation for numerous traits in various crop plants. Mason and Zuber (1976) have employed diallel cross analysis in maize breeding, while Jinks (1954) applied it to cotton. In wheat, Crumpacker and Allard (1962) used diallels in studing heading dates, and in barley, Johnson and Aksel (1959) examined yield inheritance. Heterosis was studied by Turner (1953) in upland cotton and Tan *et al.* (1979) applied diallel analysis to bromegrass. (See "Materials and Methods"

for a detailed discussion of diallel cross analysis.)

E. GENOTYPE X ENVIRONMENT INTERACTION

Herbage plants, such as alfalfa grow under a great diversity of conditions represented by variations in soil and weather conditions and management within a growing season and over the years. Thus where genotype x environment interactions exist, valid genotype comparisons can only be made within an environment but the stability of a cultivar over a range of environments may be studied (Breese, 1969). Genotype x environment interactions represent a change in the relative ranking of the genotypes and a change in the magnitude of differences between genotypes from one environment to another (Nguyen, 1980).

The most commonly used technique of analysing genotype x environment interactions is the linear regression technique where the interaction is partitioned to sources due to the interaction of each genotype with the environment (Eberhart and Russel, 1966; Finley and Wilkinson, 1963). (See "Materials and Methods" for a discussion of genotype x environment analysis.)

There are relatively few studies reported using regression analysis on perennial forage species and these include reports by: Gray (1982) and Breese (1969) in orchardgrass (*Dactylis glomerata*), Troughton (1970) and Hill and Samuel (1971) in perennial ryegrass (*Lolium perenne*), Tan *et al.* (1979) in smooth bromegrass (*Bromus inermis*) and

Nguyen *et al.* (1980) in tall fescue (*Festuca arundinaceae*). The regression technique has been more widely applied to annual crops such as maize (*Zea mais*) (Eberhart and Russel, 1966, 1969), and soybean (*Glycine max*) (Baihaki *et al.*, 1976).

The interaction of genotypes with the environment is under genetic control (Eberhart and Russel, 1966) and follows a predictable pattern which can be measured by regression and can be used as a selection trait in breeding for stable genotypes.

III. MATERIALS AND METHODS

A. INTRODUCTION

Four experiments were conducted in this study, two in the field and two in the glasshouse. The field tests consisted of two full diallels (I and II) composed of progenies from eight and nine alfalfa clones respectively. They were grown on non-alfalfa sick soil to provide a summer cut (A) in July and a fall cut (B) in September. Experiment three in the glasshouse was composed of a half diallel (III) of progenies from nine clones grown on alfalfa sick soil and the fourth experiment was a comparative study of four alfalfa strains grown in sterilised and unsterilised sick soil. Sick soil is the term given to soils which induce alfalfa sickness symptoms in seedlings as described by Webster *et al.* (1967). In the glasshouse studies, sick soil was collected from a field in Spruce Grove which was identified as causing alfalfa sickness by Faechner (1978).

In the four experiments, seed preparation prior to planting was common. Alfalfa seeds were scarified with sandpaper and germinated on moist filter paper in petri dishes at room temperature. At a radicle length of 0.5 to 1 cm, the germinated seed was inoculated with a commercial culture of *Rhizobium meliloti* and planted.

B. MATERIALS

The parental clones used in the three diallel crosses were selected by Faechner (1977) for their high yield and resistance to alfalfa sickness. Diallel I contained F, crosses and reciprocals of eight clones (Appendix 3), while diallel II compared the same progenies with commercial Beaver. All parental clones originated from cultivars of *Medicago media*. The clonal name was composed of a letter derived from the cultivar name, (Roamer (R), Grimm (G), Vernal (V) and Beaver (B)) the numbers 0, 1, 2 and 3 preceded the cultivar letter and represent the selection cycle, while the number after the cultivar name was arbitrarily given in 0 selection cycle. The parental clones were established in the glasshouse on a sandy loam and intercrossed manually in all possible combinations to yield seed which was stored in a cool environment prior to planting. The parental lines were not included in the three diallel tests. In experiment four two resistant strains 2B9 X 2G169 and Br 1 (Appendix 3), one susceptible line 1GP130 X 1V58 and Beaver were evaluated. The letter P in 1GP130 designates a line selected for its susceptibility. Br 1 is one of six alfalfa strains synthesised from Faechner's resistant selections. The constituent strains (Appendix 4) were grown in the glasshouse and intercrossed manually to give seed stock for the synthetic.

C. METHODS

FIELD EXPERIMENTS

Diallels I and II crosses were established in adjacent fields at the University Parkland Farm in Edmonton (location NE07-052-24-4), The site chosen has a black chernozem silty clay loam and was fallow for three years prior to planting. Appendix 5 gives the physical characteristics of the soil, while Appendix 6 is a summary of the rainfall and temperature data from 1975 to 1984 for the months May to September inclusive. The 10 year average rainfall is 35.2 cm with a mean daily temperature of 13.1°C.

Field preparation involved discing, harrowing and an application of a granular phosphatic fertilizer (0 45 0) at 300 kilograms per hectare (135 kg P_2O_5/ha). The germinated, inoculated seed was planted in sterilised soil in individual root trainers. Appendix 5 gives the physical characteristics of this soil. The seedlings were grown in the glasshouse at 18°C under natural lighting (April and May) for six weeks, then cut back to 10 cm from the soil. They were then moved to Parkland Farm and hardened in the sun prior to planting. At planting, one litre of a starter solution (10 52 10) at 5 grams per litre was given to each plant.

Diallel I was established in June 1982 and contains progenies of eight resistant parents. A randomised block design with three replication and four plants in each plot was employed (Griffing's method 3, mixed model II). Yield

data were obtained from six cuts. Two cuts, one in mid July (A), the other in late August (B) were made in each of the three growing seasons (1982, 1983, 1984), using hand sickles to cut each individual plant. Plants from each plot were placed in a cloth bag and dried at 35-40°C for three to five days (until they were completely dry) and then weighed to the nearest gram. Also recorded were the number of plants in each plot at the time of cutting.

Diallel II, also a randomised block design, contained progenies of nine parents (the same eight parents in diallel I and Beaver), in six replications with six plants per plot. This diallel was established in June 1983 using the same procedures described previously. Three cuts were taken, one using hand sickles in September 1983 and two using a mower and a sickle in July and August 1984. The samples were dried and weighed to the nearest gram. The six cuts from diallel I, and three cuts from diallel II constituted the nine different environments for which the Eberhart-Russel regression technique of genotype stability analysis was employed.

GLASSHOUSE EXPERIMENTS

DIALLEL III

In the glasshouse, a half diallel without the parental lines was established on alfalfa sick soil to evaluate the genetics of plant reaction to alfalfa sickness. The alfalfa sick soil is a light textured

chernozem within the black great group composed of 75 percent Peace Hills fine sandy loam and 25 percent Ponoka loam. The soil was collected from several random locations within a sick field to a depth of 15 cm at Spruce Grove, 28 km west of Edmonton. The soil was thoroughly mixed and sieved in a 0.6 cm wire mesh screen and stored in a cold room at 4°C. The thirty six crosses were grown in a randomised block design with six replications and four plants per plot. The experimental unit was a 13 cm diameter plastic pot which was filled with soil up to approximately 2.5 cm from the top. Stylofoam chips were placed beneath the soil to facilitate drainage.

Four germinated seeds were planted in each pot and weeding was done by hand. This experiment was grown for thirty five to fourty two days at a temperature of 18°C under natural spring and summer daylight (April to August 1984). Plant height, leaf area, dry leaf weight, total dry matter yield and root disease rating were determined. Plant heights were measured from the soil surface to the top leaf while leaf area was measured using an automatic leaf area meter. The leaves (in one bag) and the stems and petioles (in another bag) were oven dried at 57°C for seven to nine days until they were completely dry. The dry weights were taken to the nearest .001 g. Root disease rating was based on the system used by Faechner and Bolton (1978). The roots

were washed clean of all soil and visually examined under a light microscope and rated on a scale of 1 to 5 as follows:

1. clean healthy roots
2. roots with slight browning and lesions
3. roots with brownish well defined lesions
4. roots with severe lesioning
5. dead plants.

A rating of 5 was not recorded in this experiment as plant death could not be exclusively associated with alfalfa sickness.

EXPERIMENT FOUR

This experiment involved growing four alfalfa strains 2B29 X 2G169 (F.R.), 1GP130 X 1V58 (F.S.), Br 1 and commercial Beaver in sick and sterilised soil for a period of 35 to 42 days to compare their performance as different genetic strains in the two soil treatments. Sick soil was collected, sieved and stored as in the at 1.8 kg per square centimeter glasshouse diallel and part of it was autoclaved at 130°C for 30 minutes prior to planting, 13 cm plastic pots were filled with soil and planted with four seeds which had been germinated and inoculated with *R. meliloti* as described earlier. Each entry was grown in six pots containing sick soil and six pots with sterilised soil. This allowed for a destructive sampling of one pot from each of the sick and sterilised soil for each entry every seven to nine

days. The samples were assessed for plant height, leaf area, leaf dry weight and root necrosis. The experiment was maintained at 18°C under natural lighting from April to August 1984. A split plot design was used with the four strains and two soil treatments assigned to the main and subplots respectively.

At each sampling date, one pot of each entry was analysed from the autoclaved and unautoclaved sick soil for plant height, leaf area, leaf dry weight and root browning. From the leaf parameters it was possible to calculate the specific leaf weight so that it might be used as an index of photosynthetic activity over the growing period. Cross product analysis of these traits was conducted.

D. STATISTICS

DIALLEL CROSS ANALYSIS

The diallel cross analysis was developed by Jinks and Hayman (Jinks and Hayman, 1953; Jinks, 1954; Hayman, 1954a, 1954b) and has been modified (Griffing, 1956; Hallower, 1981) to provide an estimate of the genetic components of variation after only one filial generation. Griffing (1956) noted that parental and F_1 data have distinct advantages over data from segregating generations in studying quantitative genetic systems as they are unaffected by segregation and linkage, and therefore require relatively

few individuals for efficient estimation of genetic parameters.

A diallel is the set of P^2 possible single crosses and selfs between p homozygous lines (Hayman, 1954; Griffing, 1956). These crosses may be represented by a p x p matrix containing i) the inbred lines, ii) one group of p(p-1)/2 F_1's, iii) one group of p(p-1)/2 reciprocal F_1's.

There are four possible classes of diallel crosses depending on the inclusion or exclusion of the parents and the reciprocal F_1's. These are:

1. a full diallel where the parents, one set of F_1's and the reciprocals are included (p^2 combinations),
2. a half diallel where the parents and one set of F_1's are included (1/2 p (p+1) combinations)
3. a "full progeny" diallel where the F_1's and reciprocal F_1's are included (p(p-1) combinations)
4. a partial diallel where only one set of F_1's is included (1/2p(p-1) combinations).

Each diallel method yields different statistics and consequently the analysis and estimation of components of genetic variation vary.

Analysis also varies depending on the assumptions made pertaining to the sampling, the genetic control of the trait(s) of interest and the experimental design. Griffing (1956) identified two alternate assumptions related to the sampling of experimental material

1. the parental lines or the experimental material are a

random sample from some population about which inferences are made,

2. the parental lines are deliberately chosen and therefore the experimental material constitutes the entire population about which inferences are made.

There are six basic assumptions concerning the genetics of the experimental material in a diallel. These are:

1. diploid segregations
2. absence of reciprocal differences (maternal effects)
3. homozygosity of parental lines
4. absence of multiple allelism
5. independent gene distribution
6. independent action of non allelic genes.

Another set of assumptions relate to the experimental design with specific reference to whether the experimental material is assigned to experimental units in a fixed or random manner. The most commonly used design in diallel analysis is the randomised block design (Griffing, 1956) where the experimental material is assigned either to fixed or randomised blocks.

The sampling and design assumptions give rise to Griffing's four methods of diallel analysis.

1. Model I where the variety (material) and block effects are constant.
2. Model II where the variety and block effects are randomised.
3. Mixed Model I where the variety effects are random and

the block effects are constant.

4. Mixed Model II where the variety effects are constant and the block effects are random.

The diallels in this study are mixed model II, methods 3 and 4 for the field and glasshouse diallels respectively. Experimental populations rarely meet all the assumptions but forms of diallel analysis have been developed to provide a measure of the deviations from some of the assumptions. Allelic interactions and reciprocal effects may be estimated and the condition of homozygosity may be waived if there is sufficient genetic variability among the parental lines, with a similar coefficient of inbreeding (Hayman, 1954; Kempthorne, 1956).

In general, diallels can yield the following estimates

1. the total phenotypic variation
2. the genetic and environmental component of variation
3. the additive and non additive components of genetic variation
4. the dominance and epistatic interactions.

Griffing's approach of employing general and specific combining abilities to estimate genetic parameters has been used here. The general combining ability (G.C.A.) has been defined as the average performance of a line in hybrid combination and the specific combining ability (S.C.A.) refers to those hybrid combinations which are above or below the average performance of a give line (Sprague and Tatum, 1942). The additive and non-additive components of genetic

variation is estimated from their direct relationship to the G.C.A. and S.C.A. respectively since:

$\sigma a^2 = 2\sigma g^2$ and $\sigma na^2 = \sigma s^2$

where

σa^2=additive genetic variance

σna^2=non-additive genetic variance

σg^2=G.C.A.

σs^2=S.C.A.

The estimation of these components of genetic variance however is only possible in experimental material which meet the assumptions adequately. Alfalfa is an autotetraploid and because it forms diploid gametes does not reach equilibrium after one generation of random mating. The parental lines are not homozygous and the results from these experiments can only be used to allude to the relative importance of additive and non-additive genetic variation in the system.

Griffing's analysis of diallel crosses consists of three basic steps:

1. an analysis of variance for genotypic differences among the entries in the experiment and a test of the effectiveness of the experimental design,
2. an analysis of variance for the reciprocal and maternal effects and the general and specific combining abilities of the inbred (parental) lines,
3. an estimation of the general and specific combining ability effects.

This provides a quantitative measure with which parental lines and their progenies may be evaluated. An estimation of the genetic components of variation may constitute a fourth step provided the assumptions are appropriately met.

Diallels may be incorporated into a variety of breeding programmes to develop hybrid or synthetic cultivars by means of recurrent or mass selection. The diallel selective mating system (Hallower, 1981) was developed as a means of broadening the genetic base of the breeding population particularly in autogamous crops. It enables the intercrossing of selected plants in each generation for maximum recombination, and new germplasm may be introduced at any stage of selection, with cultivars being extracted at any stage.

STABILITY ANALYSIS

The development of forage cultivars which maintain a high level of performance over a wide range of environments is an important goal of most breeding programmes. The relative performance of different genotypes varies from one environment to another for quantitative traits such as yield. This necessitates the study of genotypes by environment (G X E) interactions which, if not evaluated detract from the efficiency of the selection procedure while making it difficult to decide on the most suitable genotypes.

The classical approach to studying G X E interactions (Sprague and Federer, 1951) estimates the variance due to the environments and the G X E interaction but gives no measure of individual genotype response to different environments (Nguyen *et al.*, 1980). This response is determined in the linear regression analysis technique which was originally proposed by Yates and Cochran (1938) and later modified by Finley and Wilkinson (1963) and Eberhart and Russel (1966). The Finley and Wilkinson linear regression technique provides one measure of stability, the coefficient of regression (*bi*) of cultivar mean at each environment on the mean yield of that environment. The Eberthart and Russel technique provides two stability parameters; the regression coefficient obtained by the regression of an environmental index (measured by the mean performance of all genotypes grown in a given environment minus the grand mean) on the performance of each genotype in each environment, and the deviation from regression mean square (Sd^2). The following model defines the parameters used in the Eberhart-Russel regression analysis technique:

$$\gamma_{ij} = \mu_i + \beta_i + I_j + \delta_{ij}$$

where γ_{ij} is the variety mean of the *i*th variety at the *j*th environment, μ_i is the mean of the *i*th variety over all environments, β_i is the regression coefficient that measures

the response of the *i*th variety to varying environments, δ_{ij} is the deviation from regression of the *i*th variety at the *j*th environment, and I_j is the environmental index obtained as the mean of all varieties at the *j*th environment minus the grand mean. In this model, a stable genotype is one with a unit regression coefficient ($bi=1.0$) and with no deviations from regression ($S^2d=0$). Finley and Wilkinson (1966) defined stable cultivars as those whose performance was relatively constant over different environments with a regression coefficient below 1 ($bi<1.0$). This definition was criticised by Breese (1969) who stressed the importance of deviations from regression in assessing stability and by Eberhart and Russel (1966) who established that cultivars with a regression coefficient less than 1 often have below average mean yields. The complete analysis of genotype stability involves an analysis of variance for the genotype and environments and genotype x environment interaction, followed by the linear regression analysis. The linear regression technique can be used to predict the performance of genotypes in environments other than those sampled experimentally (Tan *et al.*, 1979).

IV. RESULTS

A. DIALLELS I AND II

An analysis of variance for dry matter yield was conducted on data from diallels I and II for each of the nine cuts for seventy-two crosses (Table 1). There were significant genotypic differences in cuts A and B in 1982 and 1984 for diallel I and in 1983, and cuts A and B of 1984 for diallel II. Significant F values were obtained for the 1983 A cut but not the 1983 B cut of diallel I. The F values for total yield were highly significant for 1982 and 1984 and for the combined 1982 to 1984 analysis in diallel I. The analysis of total dry matter yield in 1983 of diallel I and 1983 and 1984 combined in diallel II were significant at $P<0.05$, while no genotypic differences were detected in the analysis of the total yield of 1984 in diallel II. Except for the establishment years, larger F ratios for genotype mean square were obtained in the cut A than the cut B.

Tables 2 and 3 present Griffing's analysis of variance among the alfalfa clones in diallels I and II for general and specific combining ability (G.C.A., S.C.A.) and for maternal and reciprocal variances. There were significant G.C.A. variances among the parental clones in all cuts of the two diallels except the 1983 cuts (A and B) of diallel I. No significant S.C.A. variances were observed in any cut except 1982 A of diallel I where they were significant only at $P<0.05$. Reciprocal differences among the clones were

predominantly due to maternal effects in diallel I and were detected only in the 1983 cut of diallel II. The test for reciprocal maternal differences was not significant in 1984 A and B of diallel II and 1983 B of diallel I. With the removal of the maternal effects, the residual reciprocal differences among the nine clones and their progeny were insignificant.

In Table 4, the G.C.A. effects of each clone at each environment are presented with the standard error of difference between any two effects. Table 5 summarises these results, listing the clones that were significantly different from each other at each environment, and over the nine environments. Parental clones 2G169, 1VP9 and 2B76 had predominantly high effects while 2V13 and 2B183 had predominantly low effects. Clone 2B29 had G.C.A. effects closest to the mean while 1G169 and 2R187 exhibited inconsistent effects in the two diallels. Clone 1G169 had high effects in diallel I and low effects in diallel II while 2R187 exhibited the reverse situation. Beaver had G.C.A. effects close to the mean in diallel II.

An overall ranking of the clones for G.C.A. in the two diallels placed clones 1VP9, 2G169, 2B76 consistently at the top, 2V13, 2B183 at the bottom and 2B29 in the middle. Clones 1G169 and 2R187 could not be classified in this manner due to their apparent lack of stability for G.C.A. in the two diallels. The differences observed in the relative clone performance over the nine environments pointed to the

need for genotype stability analysis.

Differences in clone performance as male or female parents have been listed in Table 6 to give a measure of maternal effects. In diallel II, maternal effects were not detected but in diallel I they were present. Overall maternal effects were negative for clones with high G.C.A. (2G169, 1VP9, 2B76, 1G169). Clones 2R187 had a relatively small G.C.A. effect and portrayed negative maternal effects while the rest of the clones with average of low G.C.A. (2B183, 2V13, 2B29) had positive maternal effects.

B. GENOTYPE STABLILITY ANALYSIS

The Eberhart-Russel regression technique for stability analysis was applied to eight alfalfa clones by using the individual dry matter yield for the progeny of each clone over the nine environments in diallels I and II as a basis for comparison. Progenies from Beaver were excluded from this analysis as they were not grown in diallel I.

The analysis of variance for environmental, genotypic and genotype by environment interaction effects (Table 7) revealed differences among the nine environments, and significantly different general and specific combining abilities of the parental clones. In this combined analysis for diallels I and II, the variance due to S.C.A. effects was observed while it was not significant in individual cuts (Tables 2 and 3). This may be attributed to the increased error degrees of freedom in the combined analysis of

variance. The mean square ratio of the G.C.A. to S.C.A. was 2.50 indicating the predominance of G.C.A. effects in the combined analysis of variance inspite of the significant S.C.A. variances. Genotype by environment interactions were significant at P=0.05.

Table 8 shows differences in mean dry matter yield at each environment. The differences arise from the relatively low dry matter yield at the first cuts after establishment (1982 A diallel I, 1983 diallel II) in conjunction with the relatively low yields of the second cuts compared to the first cuts in the harvests of 1983 and 1984 for diallel I and 1984 for diallel II. The average mean dry matter yield of the environments was 3965 kg/ha and 3651 kg/ha for diallels I and II respectively. Differences of 2828, 1720, and 3314 kg/ha existed between the first and second cuts in 1983 and 1984 for diallel I and in 1984 of diallel II.

Table 8 also presents the mean clone performance of each environment in terms of the Eberhart-Russel environmental index for which the average is zero. These indices were regressed with the mean clone dry matter yield at each environment (Table 9) to obtain two stability parameters, a regression coefficient *b*i and mean square deviations from regression for each clone. Table 10 shows that there was a significant linear relationship between mean genotype preformance and the environment for all parental clones except 1VP9 which has a regression coefficient value of 0.58. Clone 2R187 had *b*i=0.67 while the

other clones had regression coefficients between 0.88 and 1.17. The amount of variation accounted for by the regression analysis ranged from 95 to 100% except 2R187 which had a significant R^2 value of 0.82, and 1VP9 with a non significant R^2 value of 0.34.

In an attempt to obtain a significant relationship between mean clone dry matter yield and the environment for 1VP9, the mean clone yields for all clones were transformed to $\log_{10}$ and regressed against the Eberhart-Russel environmental index (Table 11). A significant linear relationship of clone performance to the environment was attained for all clones with R^2 values of 0.81 for clone 2R187 and values between 0.91 and 0.98 for the remaining clones, including 1VP9. The $\log_{10}$ transformations reduced the variation between the stability parameters of the different clones and induced a high degree of linearity for 1VP9.

C. DIALLEL III

Among the thirty-six crosses grown in the glasshouse in diallel III, significant differences were detected for plant height, and root necrosis but not for leaf area, leaf dry weight, stem weight, total dry weight and specific leaf weight (Table 12). There were also highly significant differences among the six replications for all characters. Griffing's analysis of variance for general and specific combining ability reveal G.C.A. differences for plant height

and leaf area but not for leaf dry weight, specific leaf weight, and root necrosis. The S.C.A. variances were not significant for any of the traits analysed (Table 13).

Cross product analysis revealed there was a positive G.C.A. for:

1. Plant height with leaf area, stem dry weight, and specific leaf area but not with leaf dry weight and total dry weight;
2. Leaf area with stem dry weight and root necrosis;
3. Stem dry weight with all the traits except leaf weight;
4. Leaf dry weight with specific leaf weight (Table 14).

The analysis also showed that total dry matter had a negative G.C.A. relationship with root necrosis.

G.C.A. effects have been presented for plant height and leaf area where significant differences were detected (Table 15). For plant height, clones 1VP9 and Beaver had the lowest G.C.A. effects and differed from 2B183 and 2B187 which had high G.C.A., but did not differ from the other five clones. Low G.C.A. effects for leaf area were observed in 1VP9, 2B29, 2B76 and Beaver. These clones were not significantly different from each other but differed from the remaining clones. Clones 2V13 and 1G169 but were different from 1G169 to 2G169 and 2B187. Clone 2B183 had the highest G.C.A. effect for leaf area which differed from those of all other clones.

D. EXPERIMENT FOUR

Analysis of variance for plant height, leaf area, leaf dry weight, specific leaf weight and root necrosis of four alfalfa cultivars grown in sterilised and unsterilised sick soil over six weeks revealed significant differences among genotypes, between the two soil types and over all traits (Table 16). Significant genotype x soil interactions were observed for height, specific leaf weight, and root necrosis but not for leaf dry weight and leaf area. Genotype x time interactions were significant for plant height, leaf area, and disease but not for leaf dry weight and specific leaf weight. The three way interactions of genotype x soil x time was significant for leaf area and disease score.

At the last sampling (Table 17) Faechner's resistant line 2B29 x 2G169 (F.R.) was taller than Beaver and Br 1 which were in turn taller than Faechner's susceptible line 1GP130 x 1VP58 (F.S.). In sterilised sick soil Br 1 and Beaver were not significantly different from each other. In the unsterilised sick soil F.R. was taller than Br 1 which was significantly taller than Beaver, while Beaver was taller than F.S. These significant differences were established by week four and increased with time (Table 18). Plant height increased exponentially in both soil types and was greater in sterilised than unsterilised sick soil from week four for all genotypes. The two-way interactions of genotypes with time and genotype with soil were observed in the early growth stages (weeks 2 and 3).

In week seven the largest leaf area in the sterilised soil was observed for F.R. It was not significantly different from that of Br 1, but it was different from Beaver and F.S. (Table 17). At this stage Br 1 and Beaver had similar leaf areas which were larger than that of F.S. In unsterilised sick soil, (Table 18), at the last sampling F.R. and Br 1 had similar leaf areas, larger than Beaver's which was in turn different from the low leaf area of F.S. Table 18 shows that interactions between genotypes, soil and time occurred predominantly in the first three weeks of plant growth. The average leaf area was significantly greater in sterilised than unsterilised soil.

At the last sampling in the sterilised soil (Table 17) leaf weights were greatest for F.R. followed by Br 1, Beaver, and F.S. in that order and were all significantly different from each other. In the unsterilised soil Br 1 had the highest leaf weight followed by F.R., Beaver and F.S. in that order, all significantly different from each other. Genotypic differences for leaf weight were observed from week four (Table 18), and in all genotypes higher weights were observed in the sterilised than unsterilised soil.

In week seven there were no significant differences in specific leaf weights (S.L.W.) for all genotypes in sterilised sick soil, and in the unsterilised sick soil (Table 17). F.R. had a higher S.L.W. than Beaver, Br 1, and F.S. which were not significantly different from each other. The S.L.W. in week two were high (Table 18).

With respect to root necrosis (Table 19) there were no significant genotypic differences in weeks two and three, but F.S. and F.R. showed valid differences by week four. In week five all genotypes were significantly different for root necrosis with F.S. having the highest disease score (2.9) followed by Beaver (2.4), Br 1 (1.8) and F.R. (1.4). In the following week F.S. had a significantly higher disease score than Beaver, Br 1, and F.R., and Beaver was different from F.R. while F.R. and Br 1 were similar. F.S. was more necrotic than the other genotypes which exhibited similar root necrosis in week seven. For F.S. and F.R., the disease scores increased up to week six and decreased in week seven. Scores also decreased from week six for Beaver. For Br 1, the disease score increased during the first four weeks then dropped in week five and rose again in weeks six and seven. Overall root disease score rose to weeks five to six from where it was seen to plateau. F.R. exhibited the least disease symptoms followed by Br 1 and Beaver, while F.S. had the most root disease.

V. DISCUSSION

In this section, the results will be discussed bearing in mind that since the parental clones of the three diallels (except Beaver) had previously been selected for resistance to alfalfa sickness, the experimental material does not represent alfalfa populations in general. Therefore, the results pertain only to the clones in the experiment. The scope of the study was broadened by experiment four in which alfalfa sickness resistant strains as represented by F.R. and Br 1 were compared to susceptible strains (F.S.) in sterilised and unsterilised sick soil.

A. DIALLELS I and II

Analysis of dry matter yield data from diallels I and II, conducted in the field under nine different environments enabled the classification of the alfalfa clones with respect to dry matter yield, general combining ability and stability.

The progenies of the clones exhibited significant genotypic differences at all except the 1983 cut of diallel I. At that environment, the tests for general and specific combining abilities as well as the additive reciprocal effects (maternal) and specific reciprocal effects of the clones were not significant. This suggests that there was an overriding influence of factors not studied in these tests in the 1983 B cut for diallel I. These factors have not reduced the mean yield and could not be determined. The

absence of significant S.C.A. variances show that non-additive gene action was not an important factor in controlling dry matter yield differences among the alfalfa clones. S.C.A. differences were only detected in the first cut of 1982 in diallel I at P=0.05. Busbice (1969) indicated that non-additive genetic variance had a very small effect on differences among alfalfa synthetics with more than four parents since the variance among synthetics decreases rapidly with an increasing number of parental lines. The presence of significant G.C.A. (Tables 2 and 3) indicates that additive gene action was an important factor determining dry matter yield among the alfalfa clones. A positive response to recurrent selection could therefore be expected particularly if clones 1VP9, 2G169, and 2B76 which portrayed high G.C.A. effects in most environments were used. The inclusion of clone 2B29, with near zero G.C.A. effects, as a fourth parent in the selection would serve to maintain a sufficiently broad genetic base. Busbice (1969) suggested that at least four parents were necessary to prevent excessive inbreeding in the advanced generations of a synthetic. Response to selection would be enhanced with the removal of clones 2V13 and 2B183 which had low G.C.A. effects. The clones with inconsistent G.C.A. effects between the two diallels, 1G169 and 2R187 indicated the need for a study of traits other than G.C.A.

Maternal sources of variation have been shown to be important in determining seedling characters (Cal and

Obendorf, 1972; Carnahan, 1963; Singh and Hadley, 1972), but not in determining mature plant characters (Van Sanford and Matzinger, 1982).

The importance of G.C.A. in developing synthetics with good spring growth, regrowth after cutting, dry matter yield and resistance to disease has been reported. Busbice *et al.* (1972) stressed the importance of maintaining a broad genetic base in the synthetic since alfalfa is very sensitive to inbreeding depression even at low levels. Successive generations of self fertilization are accompanied by a pronounced reduction in vegetative vigour and seed yield due to the genetic load of undesirable factors (Busbice, 1969).

Both S.C.A. and G.C.A. are important in crops like maize where hybrid cultivars are commonly used (Crumpacker and Allard, 1962; Griffing, 1956; Jinks and Hayman, 1953; Mason and Zuber, 1976; Rutger *et al.*, 1971). There is evidence of heterosis in alfalfa (Busbice, 1969), and in order to utilise non-additive genetic variance (S.C.A.) alfalfa breeders could resort to the production of narrow based synthetics using inbred lines from which the undesirable factors has been removed by selection. Hybrid cultivars of alfalfa are not common due to difficulties in seed production as male sterile plants are not attractive to insects.

B. STABILITY ANALYSIS

The ability of a genotype to respond to a change in environment is under genetic control (Eberhart and Russel, 1969), and therefore plant selection for stability of performance over varying environments is possible. Variations among the nine environments occurred due to differences in climate, the effect of cutting, and the stage and age of plant growth within a growing season and over the years. Different locations, years, management practices and various combinations of these factors have been used to represent different environments in genotype x environment studies of various crops. Tan (1979) used different locations chosen to provide differences in soil type, annual precipitation and temperature within Alberta over two years for spring and fall cuts of bromegrass. Nguyen (1980) used locations and years while Gray (1982) also varied the environments by differences in plant spacings. In this study, a larger genotype x environment interaction could have been obtained if the experiments had been conducted at more than one location.

In this study the first cut after estabishment in each diallel was low yielding, and in subsequent years, the attainment of 10 percent bloom (harvesting time) in the second cut was accompanied by less dry matter accumulation in comparison with the first cut. This summer decline in dry matter accumulation for alfalfa has been reported in the literature (Bula and Messengale, 1972) and associated

primarily with the higher temperatures and longer day length of this season. High soil temperature may inhibit symbiotic nitrogen fixation. Higher temperatures and longer daylight hours result in rapid development towards reproduction and is accompanied by a reduction in vegetative growth (Bula and Messengale, 1972). Lower rainfall also contributes to the reduced yields of the fall cuts.

The environments were quantified on the basis of the mean yield of all genotypes at that environment, and this method of classification has been widely used (Eberhart and Russel, 1966, 1969; Finley and Wilkinson, 1963; Gray, 1982; Nguyen, 1980; Tan *et al.* 1979). Tan *et al.* (1979) assessed their environments as the mean expression of a) all genotypes and b) the parental clones at a given environment. Hill and Baylor (1983) also used the mean of all genotypes except the one for which the regression was being analysed.

Classifying environments on the basis of the mean performance of the genotypes at that environment introduces a bias due to the genotypes in the test (Tan *et al.*, 1979). Breese (1969) compared this type of environment classification to quantifying genotypes by their average expression over a range of environments, and as the average genotype value is influenced by the environments, so is the average environment value influenced by genotypes grown in it. To reduce the genotype bias in classifying environments, Hill (1975) suggested the use of extra replications of the full set of genotypes.

Significant G.C.A. and S.C.A. variances and the interaction of G.C.A. with the environment were detected in the analysis of variance (Table 7). The G.C.A. variance was more significant than G.C.A. x environment interaction indicating the greater effect of additive gene action over its interaction with the environment.

The genotype x environment interaction among the alfalfa clones was explained by means of the regression coefficient, as was done by Gray (1982). This author referred to the regression coefficient as a performance "response index" (Gray, 1982), measuring response to a changing environment. Clone 1VP9 exhibited significant changes in the magnitude of differences from the other clones from one environment to the other. On the whole, the other five clones (2G169, 2B76, 2V13, 2B183 and 2B29) ranked in the same order with small changes in the magnitude of differences from one environment to the other. Thus most or all of the genotype x environment interaction could be attributed to clones 1VP9, 2R187 and 1G169.

None of the regression coefficients (except 1VP9) were significantly different from 1. The relatively small variability amongst the regression coefficients reflected the homogenous nature of the experimental material. Seven clones had undergone one and two cycles of phenotypic recurrent selection for resistance to alfalfa sickness while 1VP9 was selected for susceptibility to the sickness. However 1VP9 was included in this test on the basis of its

high yielding ability in the field. (Faechner, 1977).

The relationship between 1VP9 and the environments was not linear (R^2=0.34) unless log transformation of mean genotype yield was used. Then the relationship was significant with an R^2 value of 0.96. Finley and Wilkinson (1963), and Tan *et al*. (1979) also used $\log_{10}$ transformed mean genotype yields. For this trial an average R^2 of 0.97 was obtained for the remaining clones. Comparable R^2 values were obtained by Nguyen (1980) and Gray (1982) in tall fescue (R^2=.94) and orchardgrass (R^2=.76). These high R^2 values illustrate the fact that differences from one environment to the other follow an orderly pattern. The second Eberhart-Russel stability parameter is the deviation mean square which has been referred to as the true stability index (Eberhart and Russel, 1969; Gray, 1982). Except for clone 1VP9, deviation mean squares did not differ significantly from zero, again reflecting the homogeneity of the experimental material. The same conclusion was reached by Gray (1982) who studied genotype stability in tall fescue, while Breese (1969), and Tan *et al*. (1979) reported significant mean square deviations in orchardgrass and bromegrass.

The stability parameters (b_i and Sd^2) provided additional selection criteria and the alfalfa clones were classified on the basis of mean yields, G.C.A., b_i and Sd^2. A significant distinction among the clones could be made between 1VP9 and the remaining clones due to its

non-significant linear regression. Table 9 shows that clone 1VP9 had the highest mean yield among all the clones with just above average mean yields in the low and high yielding environments (1982 A, 1983 B, 1984 B diallel I, 1984 B diallel II; 1983 A diallel I and 1984 A diallel II) and very high yields in the medium yielding environments (1982 B and 1984 A diallel I; 1984 A diallel II). The significant R^2 value and non-significant mean squares deviation using log transformed mean yields of 1VP9 indicates that this pattern is real and predictable with a low variation. This clone would thus be best suited for production in medium yielding environments, where it would give very high yields.

Breese (1969) classified genotypes with *bi* greater than zero as being adapted to high yielding environments, and *bi* less than zero to low yielding environments. In this context, the clones were evaluated with due consideration given to mean yield, and general combining ability. Clones 2G169 and 2B76 commonly had *bi* greater than 1 with high yields and G.C.A. While clones 2B183 and 2V13 had *bi* less than 1, low yields and G.C.A. and clone 2B29 had average *bi*, yield and G.C.A. Clone 2B29 also had the lowest mean square deviation. Different relationships were observed for the mean yield, G.C.A., *bi* and Sd^2 of clones 2R187 and 1G169. Clone 2R187 had a high G.C.A., a low mean yield, and a low *bi* (0.67) while clone 1G169 had average G.C.A., mean yield and a *bi* value of 0.88.

Except for clone 2R187, high G.C.A. effects were accompanied by high mean yields and vice versa, and there was a positive relationship between the yielding ability of a clone and the capacity to respond to improved environmental conditions (*b*i). Gray (1982) also found a positive relationship between mean yield, G.C.A. and *b*i in orchardgrass, and Eberhart and Russel (1966) found that maize hybrids with regression coefficients less than 1 usually had mean yields below the average. Gray (1982) however found no relationship between mean yield and mean square deviation and this has also been observed among the alfalfa clones in the present experiment. It is difficult to decide which criteria, yield, G.C.A., *b*i, or Sd^2 is the most important. All these factors would be important in the production of a high yielding synthetic clone with a good adaptation to Western Canada, particularly north and central Alberta where alfalfa sickness has been observed.

To produce a synthetic from the eight clones evaluated (Table 10), clones 2B76, 2G169 and 1VP9 which had positive attributes for all criteria evaluated would be the most suitable parents. Clone 2B29 may also be included to maintain a wide genetic base. Further testing would be required, perhaps at different locations in order to classify clones 1G169 and 2R187, and on the basis of results from this study, clones 2V13 and 2B183 would not be suitable parents.

C. DIALLEL III

The results from diallel III grown in the glasshouse reflected the homogeneity of the clonal material. Of the seven traits evaluated, genotypic differences among the thirty six progenies were detected only for plant height and root necrosis. G.C.A. variances were significant only for plant height and leaf area. Adams and Semeniuk (1958) stated that it was possible to deplete additive genetic variance in one cycle of recurrent selection. Faechner (1977) alluded to the decreasing additive genetic component of variation for height and root necrosis in the second and third cycles of recurrent selection. Also when he evaluated all lines selected for resistance and susceptibility to alfalfa sickness in the field, the highest yielding lines were from selection cycles one and two. Strains from cycle three did not give enhanced yield. It can be concluded that significant additive variation for traits employed in selecting resistant and susceptible strains for alfalfa sickness has been depleted in the population. This points to the need to intensify selection pressure by isolating and identifying the pathogen or pathogens causing alfalfa sickness, and defining the environment which will enhance the sickness. These factors may be applied to experimental populations to provide a more precise screening for alfalfa sickness. Thus a concise definition of alfalfa sickness, its causal pathogen(s) and favoured environments is a necessary prerequisite for progress in selecting alfalfa genotypes

with increased resistance to the sickness.

The study of gene action for plant response to alfalfa sickness requires a wider genetic base of the material, so that significant genetic variances may be detected in sick soil. Furthermore, since alfalfa is an autotetraploid, at least two generations of random mating are required for complete segregation and expression of all possible genotypes. This would increase the chances of picking out segregants with high resistance to alfalfa sickness. Genetic studies would also be enhanced by the application of the precise stress which cause alfalfa sickness again pointing to a need for pathological studies.

The positive association between leaf area, leaf weight, and total dry weight to plant height as indicated by the analysis of cross products, imply that in selecting for plant height, there was indirect selection for leaf area, leaf weight and total weight. Thus there are genes commonly controlling the expression of these traits. The genetic variation for these traits was simultaneously reduced and this is reflected in the absence of G.C.A. and S.C.A. for leaf weight, stem weight, and total dry weight. The negative relationship of plant height, leaf area, leaf weight, total dry weight and specific leaf weight (which is associated with net assimilation) to root necrosis was only detected for total weight while leaf area had a significant positive relationship with root necrosis. In order to study the precise relationship of the traits representing net

assimilation rates to root disease symptoms, a greater genetic diversity of alfalfa genotypes would be required representing both resistant and susceptible genotypes. This diversity was absent in the materials used in this study.

D. EXPERIMENT FOUR

The results from experiment four may be taken to indicate that selections for resistance to alfalfa sickness based on plant height and root necrosis really represents selection for superior net assimilation rates. Components of net assimilation rate include larger photosynthetic area (increased number area and weight of leaves) and increase photosynthetic efficiency (Barnes *et al.*, 1969; Pearce *et al.*, 1969). Tan (1977) found two alfalfa sickness resistant genotypes to be high yielding for dry matter production and nitrogen fixation. Faechner postulated that resistant genotypes had the capacity to manufacture assimilates in excess of the requirements of the bacteria (*Rhizobium meliloti*) and the parasitic alfalfa sickness pathogen so as to retain sufficient assimilates for high dry matter yield. The resistant strains, Br 1 and F.R. had significantly higher leaf areas, leaf weights and plant height than F.S., the susceptible strain, and less root necrosis. Significantly higher S.L.W. were only detected in week seven for F.R. and this may be attributed to the fact that the calculation of S.L.W. was based on mean leaf area and leaf weights of many leaves. Barnes *et al.* (1969) obtained

greater precision in measuring the specific leaf weight of individual leaflets than by using ten leaflets at a time. In the present experiment, leaflets were measured from four alfalfa plants for each sample. Pierce *et al.* (1969) found that leaf weight accounted for 64 percent of the variation in photosynthesis in alfalfa. These authors showed that the age x genotype interaction for specific leaf weight was not significant and that leaf area was under independent genetic control. It is therefore possible to select for high photosynthetic capacity in the seedling based on specific leaf weight.

Experiment four also demonstrated that alfalfa sickness was a juvenile plant disease and does not increase with plant age. Faechner (1977) alluded to this showing that plant selection for resistance to alfalfa sickness may be conducted effectively with juvenile plants. The lack of root necrosis, and increase in plant height, leaf area and leaf weight in sterilised sick soil was consistent with the observation that sterilisation of the soil was an effective method of controlling the sickness (Faechner, 1977; Webster *et al.*, 1969). Thus experiment four was effective in demonstrating expected differences between resistant and susceptible alfalfa strains and the effect of sterilised and unsterilised soil.

In the field experiments, the criteria dry matter yield, G.C.A., regression coefficient and mean square deviation may be used to make recommendations for the

development of a synthetic strain. Six synthetic strains Br 1, 2 and 3 and Le 1, 2 and 3 have been developed from Faechner's resistant lines. Appendix 4 is a listing of parental lines which make up the synthetics. Br 1 and 2, and Le 1 and 2 have been tested in the western Canada uniformity trials and on average have yields comparable to that of Beaver, with Br 1 performing slightly better than Beaver. These four synthetic strains contain only two clones in common with the parental clones in the present experiment (2V13 and 2R187) and it is recommended that these clones be excluded due to their low yields and G.C.A. The synthetic Br 3 contains all eight clones evaluated in the present test as well as 2B75, but has not been tested for dry matter yield. This is the synthetic for which conclusions may be drawn from these experiments. A synthetic strain composed of clones 2G169, 1VP9, 2B76 and 2B29 would be expected to perform better than Br 3 as these clones represent high yields with good combining abilities and environmental stability. The development of another synthetic strain is therefore recommended for testing in the western Canada uniformity tests, and its performance should exceed that of Br 1 so making it significantly higher yielding than Beaver particularly in alfalfa sick soil.

The present experiments indicate that with the existing genetic variation in the resistant lines, and the existing selection techniques, response to phenotypic recurrent selection for resistance to alfalfa sickness cannot be

expected. Thus there is a need to find new genetic variation in plant response to the sickness and to improve the selection techniques. Genetic variation may be introduced from plants which show enhanced vigour relative to other plants in an alfalfa sick field. The more critical factor, however is that of improving selection techniques and intensifying the selection pressure. This will necessitate the identification of the pathogen or pathogens and the environment which cause and enhance the sickness. These may then be applied to selection programme using the present and newly introduced material.

VI. SUMMARY

1. Significant general combining ability variances in diallels I and II indicated that additive gene action was important in the control of dry matter yield among the alfalfa clones. Therefore a positive response could be expected from recurrent selection for dry matter yield. The results showed that non-additive gene action was not an important factor.
2. General combining ability and dry matter yield were positively related for all clones except 2R187 which had a low mean yield and high G.C.A. Clones 2G169, 1VP9 and 2B76 had high dry matter yields and G.C.A., clones 2B29 and 2G169 had average G.C.A. and dry matter yield while clones 2B183 and 2V13 had low G.C.A. and dry matter yield. The analysis of combining abilities can be important in identifying clones with high mean yields and high G.C.A. for inclusion in a synthetic strain.
3. The Eberhart-Russel regression analysis revealed differences in the relative rankings of clones 2R187 and 1G169, and signficant changes in the magnitude of differences between clones 1VP9 and the other clones. Therefore clones 2R187, 1G169 and 1VP9 exhibited varying performances relative to the remaining clones in different environments. There was no evidence of genotype x environment interaction for the remaining five clones. All clones had mean square deviations which were not significantly different from 0.

4. Taking into consideration mean dry matter yield, G.C.A. and *bi*, clones 2G169, 1VP9 and 2B76 would be the most suitable for combining in a synthetic strain. Despite the significant genotype x environment interaction, the lowest yield for clone 1VP9 were not below the average of the remaining clones. Clone 2B29 with average mean yield, G.C.A. and *bi* has been recommended for inclusion in such a synthetic strain to maintain a broad genetic base and avoid the detrimental effect of inbreeding depression in advanced generations of the synthetic. The synthetic strain would be expected to yield more than Br 3 particularly in medium yielding environments where the high yield of 1VP9 could be exploited fully.
5. The glasshouse diallel demonstrated the importance of evaluating sufficiently heterogenous material when studying gene action for a given trait. The alfalfa clones had previously undergone one and two cycles of selection for resistance to alfalfa sickness and among the seven traits evaluated, only two, plant height and leaf area indicated the presence of additive genetic variation. To study gene action in controlling plant reaction to alfalfa sickness, susceptible parents should be included to provide adequate heterogeneity.
6. Diallel III also demonstrated that additive genetic variation for plant reaction to alfalfa sickness has been depleted among the alfalfa clones studied. Therefore, a response to phenotypic recurrent selection

for decreased root necrosis should not be expected. Genetic variability may be introduced to the existing experimental material from vigourous plants in alfalfa sick soil. In addition, the selection pressure in the breeding programme needs to be increased.

7. Experiment four illustrated that previous selection for resistance to alfalfa sickness resulted in selecting for increased leaf area and leaf weight and therefore increased net assimilation rate might be expected. Resistant strains (Br 1 and F.R.) exhibited higher leaf area, leaf weight which represents the photosynthetic capacity of a plant. Compared to the susceptible strain (F.S.) they were taller with fewer root lesions. Thus phenotypic selection has been effective in increasing resistance to the sickness.
8. Further research in studying gene action and increasing the resistance of strains to alfalfa sickness is now dependent on a more precise definition of the sickness. The synthesis of a new strain from parental clones 2G169, 1VP9, 2B76 and 2B29 is recommended for evaluation in the western Canada uniformity trials together with Br 1. The yield of the new strain is expected to be more than that of Beaver particularly in alfalfa sick soil and resistant synthetics may on this basis be developed for licencing.
9. This study had enabled us to reach our objectives. Parents with superior G.C.A. have been identified and

may be employed in subsequent crossings and selection. All clones were relatively stable and the study identified differences in response patterns over the nine environmehts. Finally, the study of different morphological traits indicated that previous selection was indirectly selecting for traits positively associated with net assimilation rate, and genetic variability has been depleted thus future breeding programmes must widen the genetic base and increase the selection pressure. A more precise definition of alfalfa sickness is required.

Table 1 Analysis of variance for dry matter yield of forage at two harvest dates in three years from diallel I and two years from diallel II

	Mean square due to			F values for
	Replication	Genotype	Error	Genotype
Diallel I 8x8				
d.f	2.00	35.00	110.00	
1982A	8172.38	18398.44	5203.76	3.54**
1982B	15719.16	15076.84	4966.09	3.04**
1982 Total	1234.36	63834.25	18146.34	3.52**
1983A	43100.11	25650.64	17148.92	1.50*
1983B	7215.04	6859.79	5276.03	1.30
1983 Total	28872.32	47796.35	27930.12	1.71*
1984A	16724.18	33028.04	10820.99	3.05**
1984B	7664.04	8070.88	26237.71	1.94**
1984 Total	46776.45	70479.78	26237.71	2.67**
1982-84 Total	126959.83	281434.65	131353.35	2.14**
Diallel II 9x9				
d.f	5.00	71.00	355.00	
1983A	430004.50	17542.02	10496.70	1.67**
1984A	207772.63	62145.37	24624.95	2.53**
1984B	15678.97	10758.73	4807.92	2.24**
1984 Total	58357.00	252397.49	207202.83	1.22
1983-84 Total	284876.30	300202.07	231228.57	1.31*

*, **, F values significant at P<0.05 and 0.01 respectively.

table 2. Analysis of variance for the combining ability of eight alfalfa clones for diallel I, 1982-1984.

Source		Reciprocal	Maternal	G.C.A.	S.C.A.	Error	G.C.A.:S.C.A.
df		21.00	7.00	7.00	20.00	110.00	
1982A	M.S.	14774.00	40452.00	41824.00	24810.00	14157.00	1.71
	F.	1.04	2.85**	3.00**	1.75*		
1982B	M.S.	8240.00	58688.00	20899.00	4966.00	4966.00	4.20
	F.	1.65*	11.82**	4.21**	1.00		
1982Total	M.S.	32862.00	184247.00	112838.00	43140.00	22385.00	2.62
	F.	1.46	8.20**	5.04**	1.93		
1983A	M.S.	22126.00	61727.00	28964.00	15864.00	17148.00	1.83
	F.	1.19	3.60**	1.69	0.93		
1983B	M.S.	5452.00	8118.00	9714.00	6895.00	5275.00	0.45
	F.	1.03	1.54	1.84	1.31		
1983Total	M.S.	40972.00	95237.00	46096.00	38949.00	27929.00	0.08
	F.	1.46	3.41**	1.65	1.39		
1984A	M.S.	13726.00	133988.00	46119.00	13379.00	10821.00	3.45
	F.	1.26	9.76**	4.26**	1.24		
1984B	M.S.	3835.00	24312.00	9820.00	6221.00	4152.00	1.58
	F.	0.92	5.86**	2.37*	1.50		
1984Total	M.S.	28525.00	268900.00	97445.00	35674.00	26237.00	2.73
	F.	1.08	10.25**	3.71**	1.34		
1982-84Total	M.S.	195052.00	433212.00	628596.00	182069.00	131947.00	3.45
	F.	1.50	3.28**	4.76**	1.37		

*, **, F values significant at P<0.05 and 0.01 respectively.

Table 3. Analysis of variance for the combining ability of nine alfalfa clones for diallel II, 1983-1984.

Source		Reciprocal	Maternal	G.C.A.	S.C.A.	Error	G.C.A.:S.C.A.
df		28.00	8.00	8.00	27.00	366.00	
1983Total	M.S.	9336.00	17018.00	59575.00	13884.00	10514.00	4.49
	F.	0.88	1.62*	5.66**	1.32		
1984A	M.S.	112800.00	64852.00	283269.00	123919.00	107399.00	2.29
	F.	1.05	0.60	2.64**	1.15		
1984B	M.S.	15488.00	19171.00	64298.00	24438.00	19240.00	2.63
	F.	0.80	1.00	3.34**	1.27		
1984T	M.S.	200886.00	144629.00	583863.00	238787.00	206084.00	2.45
	F.	0.97	0.70	2.83**	1.16		
1983-84Total	M.S.	230937.00	209785.00	710033.00	275850.00	230556.00	2.57
	F.	1.00	0.91	3.08**	1.20		

*,**, F values significant at P<0.05 and 0.01 respectively.

Table 4. General combining ability effects of nine alfalfa clones in two diallel crosses 1982-1984.

Diallel I	1982A	1982B	1983A	1983B	1984A	1984B	Combined
1VP9	83	463	223	342	523	321	1955
2G169	275	179	575	-316	49	50	813
2B76	320	270	93	-28	-272	-174	208
2B29	4	-142	-10	-45	129	-6	-72
1G169	295	106	103	247	411	78	1239
2V13	-262	-207	-194	74	174	132	-281
2B183	-412	-302	-568	-307	-811	-350	-2750
2R87	-303	-368	-222	33	-201	-51	-111
S.E.	599	355	660	584	525	325	1831
Diallel II							
1VP9				192	467	-21	639
2G169				-203	342	151	290
2B76				162	192	134	489
2B29				19	-128	-115	-224
1G169				-105	-789	-302	-1197
2V13				-389	14	-127	-502
2B183				44	-653	54	-555
2R187				313	308	263	884
Beaver				-34	246	-36	176
S.E.				322	1019	431	1494

Table 5. Clones which are significantly different from each other with respect to general combining abilities at each environment in diallels I and II.

Environment	Significantly low	Significantly high
Diallel I		
1982A	2B183	2B76, 1G169, 2G169
	2R187	2B76, 1G169
1982B	2R187, 2V13, 2B183	2B76, 1VP9, 1G169, 2G169
	2B29	2B76, 1VP9
1983A	2B183	2B76, 1VP9, 1G169, 2G169
	2R187, 2V13	2G169
1983B	2G169, 2B183	1VP9
1984A	2B183*	All clones
	2B76, 2R187	1VP9, 1G169
1984B	2B183	2B29, 2V13, 1VP9, 1G169
	2B76, 2R187	1VP9
1982-84	2B183*	2B29, 2V13, 2G169, 1VP9*, 2B76
	2R187	2G169, 1G169, 1VP9*
	2V13, 2B29	1VP9*
Diallel II		
1983	2V13*	Beaver, 2B183, 1VP9, 2R187, 2B29, 2B76
	2G169	1VP9, 2R187, 2B76
	1G169, Beaver	2R187
1984A	1G169	2G169, 1VP9, 2R187, Beaver
	2B183	1VP9
1984B	1G169	2G169, 2R187, 2B76
1983-84	1G169	1VP9, 2R187, 2B76

* significantly different from zero.

Table 6. Maternal effects of nine alfalfa clones in two diallel crosses 1982-1984

	1982A	1982B	1983A	1983B	1984A	1984B	Combined
Diallel I							
1VP9	-566	-84	-387	268	-374	-89	-1242
2G169	-1494	-1377	-199	-132	1064	361	-1777
2B76	1264	917	-43	413	-2647	-1064	-1160
2B29	1122	1231	1808	438	-52	120	1051
1G169	-1796	-1344	-1439	-744	1547	525	-3251
2V13	754	758	627	-39	1095	566	3839
2B183	3	-143	728	-352	496	283	1015
2R187	712	39	-5	70	-1129	-704	-1017
S.E.	599	355	660	584	525	325	1831
Diallel II							
1VP9				327	432	151	910
2G169				-456	402	95	41
2B76				202	-291	-32	-116
2B29				132	-91	-31	10
1G169				-288	-95	-121	504
2V13				78	791	-237	1020
2B183				-13	-75	74	-14
2R187				-112	96	11	-5
Beaver				124	-299	91	-84
S.E.				322	1019	431	1494

Table 7. Analysis of variance for 56 F_1 hybrids from eight clones grown in nine environments.

Source	Degree of freedom	Mean square	F value
Environment (E)	8	8039376.19	245.32**
G.C.A.	7	171634.11	5.32**
S.C.A.	20	68718.66	2.10**
G.C.A. x E	56	48292.29	1.47*
Error	1705	32770.99	
G.C.A.:S.C.A.	2.50		

*,** significant at $P<0.05$ and $P<0.01$ respectively.

Table 8. Trial mean for dry matter yield (kg/ha) and environmental index for nine environments.

	Mean genotype yield (kg/ha)	Eberhart-Russel Environmental index
Diallel I		
1982A	3060	-905
1982B	4164	199
1983A	5829	1864
1983B	3001	-964
1984A	4129	164
1984B	2049	-1556
Diallel II		
1983	3651	-314
1984A	6380	2415
1984B	3066	-899

Table 9. Mean yield (kg/ha) of nine alfalfa clones in nine environments.

	Diallel I						Diallel II		
	1982A	1982B	1983A	1983B	1984A	1984B	1983	1984A	1984B
Parent									
1VP9	3131	4561	6020	3295	4577	2685	3819	3646	3048
2G169	3296	4318	6746	2730	4170	2452	3473	6679	3199
2B76	3334	4396	5408	2978	3895	2260	3793	6572	3183
2B29	3063	4042	5820	2963	4239	2404	3668	6356	2966
1G169	3296	4318	6746	2730	4170	2452	3473	6679	3199
2V13	2836	3978	5663	3065	4278	2523	3311	5937	2955
2B183	2707	3906	5319	2739	3433	2109	3690	2652	3113
2R187	2800	3849	5639	3030	3956	2366	3924	4612	3296
Beaver							3621	6595	3034

Table 10. Regression coefficients of the mean yield of each of eight clones on the trial mean (*bi*), mean square deviation (Sd^2), R^2, general combining ability and forage yield from diallels I and II in nine environments.

Parent	Yield kg/ha	G.C.A.	*bi*	Sd^2	R^2
1VP9	8450	2594.50	0.58	1403360	0.34
2G169	8403	1103.00	1.17	76078	0.97
2B76	8311	697.10	1.03	31950	0.99
2B29	8085	-295.88	1.00	5161	1.00
2G169	7956	42.46	0.88	86796	0.95
2V13	7792	-783.45	0.92	32490	0.98
2B183	7720	-3305.51	0.97	6436	0.97
2R187	7551	772.21	0.67	198983	0.82

Table 11. Regression coefficients of the $\log_{10}$ mean yield of each eight alfalfa clones on the trial mean (*bi*); mean square deviation (Sd^2); R^2; and $\log_{10}$ forage yield from diallels I and II.

Parent	$\log_{10}$ Yield	*bi* (x 10^{-4})	Sd^2 (x 10^{-4})	R^2
1VP9	3.927	0.96	9.0	.96
2G169	3.924	1.14	9.8	.97
2B76	3.920	1.05	12.3	.95
2B29	3.908	1.04	5.0	.98
1G169	3.901	0.93	16.9	.91
2V13	3.892	0.97	6.6	.97
2B183	3.888	1.07	14.4	.94
2R187	3.878	0.77	28.5	.81

Table 12. Analysis of variance for 36 alfalfa genotypes grown in sick soil with respect to plant height, leaf area, leaf weight, stem weight, total dry weight, specific leaf weight (S.L.W.) and root necrosis.

Source		Genotype	Block	Error
df		35.00	5.00	175.00
Height	M.S.	35.84	770.50	23.13
	F.	1.55*	33.32**	
Leaf area	M.S.	11448.74	728254.85	9647.16
	F.	1.19	75.49**	
Leaf wt.	M.S.	0.03	4.97	0.06
	F.	1.25	82.83**	
Stem wt.	M.S.	0.10	6.00	0.07
	F.	1.44	92.71**	
Total dry wt.	M.S.	0.34	21.69	3.00
	F.	1.31	84.25**	
S.L.W.	M.S.	1.19	7.50	1.10
	F.	1.07	6.81**	
Root necrosis	M.S.	1.36	1.29	0.49
	F.	2.76**	2.65*	

*, **, F values significant at P<0.05 and 0.01 respectively.

Table 13. Analysis of variance for the general and specific combining abilities of nine alfalfa clones grown on alfalfa sick soil using plant height, leaf area, leaf weight, stem weight, total dry weight, specific leaf weight (S.L.W.) and root necrosis for evaluation

Source		G.C.A.	S.C.A.	Error
df		8.00	27.00	175.00
Height	M.S.	63.73	27.57	23.13
	F.	2.75**	1.19	
Leaf area	M.S.	19625.36	9026.03	9647.15
	F.	2.03*	0.94	
Leaf wt.	M.S.	0.58	0.79	0.60
	F.	0.98	1.32	
Stem wt.	M.S.	0.12	0.93	0.69
	F.	0.13	1.35	
Total dry wt.	M.S.	0.37	1.45	0.26
	F.	1.45	1.27	
S.L.W.	M.S.	663873355.99	685670779.48	899207276.73
	F.	0.74	0.76	
Root necrosis	M.S.	0.37	0.33	0.26
	F.	1.45	1.28	

*, **, F values significant at P<0.05 and 0.01 respectively.

Table 14. Analysis of cross products for the general combining abilities of nine alfalfa clones grown in sick soil with respect to plant height, leaf area, leaf weight, stem weight, total weight, specific leaf weight (S.L.W.) and root necrosis.

	Leaf area	Leaf wt.	Stem wt.	Total wt.	S.L.W.	Root Necrosis
Height	694.30	0.21	0.75 *	0.60	9085.16 **	0.58
G.C.A.						
Leaf area		13.42	27.49 *	23.84	82634.84	33.75*
G.C.A.						
Leaf wt.			0.81	0.14	201.18 *	0.14
G.C.A.						
Stem wt.				0.21 *	591.71 **	0.21*
G.C.A.						
Total wt.					-0.61 **	-0.38**
G.C.A.						
S.L.W.						522.13
G.C.A.						

*, **, F values significant at P<0.05 and 0.01 respectively.

Table 15. General combining ability effects of nine alfalfa clones evaluated for height and leaf area at seven weeks of age in alfalfa sick soil.

Parent	Height	Leaf area
2G169	0.13	14.84
1G169	0.56	2.01
2B183	0.98	36.44
1VP9	-1.59	-16.07
2V13	-0.02	7.46
2B187	1.13	16.16
2B29	0.70	-24.53
2B76	-0.30	-17.14
Beaver	-1.59	-19.18
S.E.	2.50	11.34

Table 16. Analysis of variance for plant height, leaf area , leaf weight, specific leaf weight (S.L.W.) and root necrosis of four alfalfa genotypes grown for six weeks on sterilised and unsterilised sick soil.

Source		Genotype (G)	Soil Type (S)	Time (T)	G X S	G X T	G X S X T
df		3.00	1.00	5 .00	15.00	5 .00	15.00
Plant height	M.S.	360.50	1279.30	5630.60	8.20	35.20	4.00
	F.	36.57**	62.17**	959.38**	6.44**	5.99 **	0.68
Leaf area	M.S.	33868.00	192280.00	1276400.00	2882.00	8792.00	54111.00
	F.	14.53**	43.53**	592.70**	1.54	4.08 *	25.13**
Leaf wt.	M.S.	0.29	1.85	5.49	0.27	0.61	0.25
	F.	7.67**	10.65**	193.98**	1.13	2.15	0.88
S.L.W.	M.S.	0.41	0.51	0.14	0.68	0.33	0.18
	F.	3.72 *	6.75**	5.26**	5.41**	1.22	6.41
Root necrosis	M.S.	2.41	65.55	2.30	2.40	0.39	0.39
	F.	16.56**	167.90**	17.99**	16.56**	3.05 *	3.05**

*, **, F values significant at $P<0.05$ and 0.01 respectively.

Table 17. Mean plant height (cm), leaf area (cm^2), leaf dry weight (mg), specific leaf weight (S.L.W.)(mg/cm^2), and disease score (D.S.) (1-5) for four alfalfa genotypes in I. sterilised sick soil and in II. unsterilised sick soil in week seven over six replications.

I Sterilised	Genotype			
Trait	Beaver	Br 1	F.S.	F.R.
Height	38.5b	38.3b	31.8c	41.2a
Leaf area	519.2b	550.2ab	447.0c	620.4a
Leaf weight	1111.4c	1168.0b	760.8d	1414.5a
S.L.W.	2.2a	2.3a	1.9a	2.4a
II Unsterilised	Genotype			
Trait	Beaver	Br 1	F.S.	F.R.
Height	28.3c	31.2b	22.3d	33.7a
Leaf area	342.3b	424.5a	266.2c	401.9a
Leaf weight	584.9c	724.9a	553.2d	657.5b
S.L.W.	2.0b	1.8b	2.2b	3.4a
D.S.	2.2b	1.9b	3.3a	2.0b

Numbers followed by the same letter in a row are not significantly different at $P<.05$.

Disease score on a score of 1-5, 1=no lesions, 5=dead plant.

Table 18. Mean I. plant height (cm), II. leaf area (cm^2, and III. leaf weight (mg) of four alfalfa genotypes grown in sterilised (S) and unsterilised (U) sick soil for each of six weeks over six replications.

I.

	Genotype							
	Beaver		Br 1		F.S.		F.R.	
Soil	S	U	S	U	S	U	S	U
Week 2	4.0	3.0	4.0	3.8	4.5	2.7	4.8	3.3
Week 3	8.2	7.5	7.7	7.3	7.8	6.3	9.2	7.8
Week 4	16.3	10.3	16.2	13.8	12.8	10.5	18.8	14.2
Week 5	20.7	15.5	22.8	17.0	17.7	12.8	24.0	18.3
Week 6	27.3	21.3	27.0	23.3	22.2	16.7	32.2	24.7
Week 7	38.5	28.3	38.3	31.2	31.8	22.3	41.2	33.7

L.S.D.=1.3

II.

	Genotype							
	Beaver		Br 1		F.S.		F.R.	
Soil	S	U	S	U	S	U	S	U
Week 2	8.6	6.6	6.5	21.4	7.0	5.3	12.6	8.2
Week 3	26.9	28.7	29.7	26.4	28.5	28.0	29.1	30.9
Week 4	61.4	39.4	66.1	50.5	52.9	39.9	78.1	52.9
Week 5	110.6	68.8	101.9	72.0	91.3	56.4	139.9	77.6
Week 6	196.9	122.7	194.5	133.0	141.5	74.0	249.7	152.3
Week 7	519.2	342.3	550.2	424.5	447.0	266.2	620.4	401.9

L.S.D. 53.21

III.

	Genotype							
	Beaver		Br 1		F.S.		F.R.	
Soil	S	U	S	U	S	U	S	U
Week 2	14.8	9.8	25.3	13.7	11.9	9.0	17.3	14.9
Week 3	58.7	47.2	51.7	46.8	45.2	34.0	55.1	53.8
Week 4	145.6	85.7	158.4	79.1	99.6	48.0	149.8	94.6
Week 5	315.9	148.0	351.8	191.6	262.8	143.1	408.5	231.3
Week 6	609.9	431.3	750.1	471.4	512.2	254.0	829.5	578.6
Week 7	1111.4	584.9	1168.0	724.9	760.8	553.2	1414.5	657.5

L.S.D. 19.17

IV.

	Genotype							
	Beaver		Br 1		F.S.		F.R.	
Soil	S	U	S	U	S	U	S	U
Week 2	3.3	1.9	4.0	3.8	2.6	3.4	2.3	3.5
Week 3	2.6	1.9	2.4	2.2	2.2	2.0	1.8	1.8
Week 4	2.7	2.4	2.9	1.7	2.2	1.4	2.4	2.4
Week 5	2.8	2.1	3.2	2.9	2.9	2.3	2.7	2.9
Week 6	2.8	3.8	3.9	2.7	3.5	3.0	3.3	3.3
Week 7	2.2	2.4	2.3	1.7	1.9	2.1	2.3	3.4

L.S.D.=0.97 at P=0.05.

Table 19. Mean disease score* of four alfalfa genotypes grown in alfalfa sick soil for each of six weeks over six replications.

	Genotype			
Week	Beaver	Br 1	F.S.	F.R.
2	1.4a	1.3a	1.2a	1.2a
3	1.6a	1.7a	1.7a	1.4a
4	1.9b	1.7b	2.3a	2.0ab
5	2.4b	1.8c	2.9a	1.4d
6	2.2b	2.0bc	3.4a	1.7c
7	2.2b	1.9b	3.3a	2.0b

* disease score on a score of 1-5, 1=no lesions 5=dead plant.

Numbers followed by the same letter in a row are not significantly different.

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Appendix 1. World area in cultivated alfalfa.

Continent	Hectares
Europe	9 363 000
North America	13 142 000
South America	7 800 000
Asia	1 323 000
Africa	174 000
Oceania	1 213 000
World Total	33 015 000

Appendix 2. Estimated areas of dehydrated and alfalfa seed production.*

	Alfalfa production (x 1000 hectares)	
Province	Dehydrated	Certified seed
Quebec	1.2	
Ontario	3.0	
Manitoba	1.5	2.3
Saskatchewan	27.8	4.0
Alberta	24.3	5.6
British Columbia	1.6	

* Agriculture Canada. 1983. Verticillium wilt of Alfalfa. Contribution 1982-8E.

Appendix 3. List of alfalfa strains in diallels I, II and III, and experiment four.

Parental clones for diallels I, II and III

2G169

1G169

2B183

1VP9

2V13

2R187

2B29

2B76

Beaver (only for diallels II and III)

Alfalfa strains in experiment 4

Beaver

2B29x2G169-resistant cross

Br 1-resistant synthetic

1GP130x1VP58-susceptible cross

Appendix 4. Parental clones of six alfalfa synthetics strains which were selected for resistance to alfalfa sickness.

Brooks 1 (Br 1)	Brooks 2 (Br 2)	Brooks 3 (Br 3)	Lethbridge 1 (Le 1)	Lethbridge 2 (Le 2)	Lethbridge 3 (Le 3)
2V13	2V13	1G169	2V13	1B29	1G169
1B70	1B70	2G169	1B29	1B110	2G169
1B29	1B24	1VP9	1B110		
1B110	1R187	2B183			
1B24		2B29			
2R187		2B75			
		2B76			
		2V13			
		2R187			

Appendix 5. Soil characteristics of samples taken from sterilised and unsterilised alfalfa sick soil (Spruce Grove) and Parkland farm*, 1984.

	pH	Available nutrients (lb/acre)			Soil conductivity (mmhos/cm)
		N	P	K	
Ster. sick soil	6.2	78	146	606	0.6
Unster. sick soil	6.2	70	116	650	0.5
Parkland farm	6.1	60	8	492	0.6

* From Soil and Feed Testing Laboratory, O.S. Longman Bldg., Alberta Agriculture, Edmonton, Alberta.

Appendix 6. Weather data for the field site at Parkland farm for diallels I and II, from 1975 to 1984*.

Year	Rainfall (mm)	Mean daily temperature °C	Daily temperature °C	
			Maximum	Minimum
1975	302.1	12.3	17.6	7.0
1976	308.2	14.1	20.1	8.0
1977	416.2	13.0	18.8	7.2
1978	495.0	13.1	18.6	7.6
1979	348.0	12.5	18.1	6.9
1980	397.1	13.4	19.1	7.6
1981	285.8	14.1	20.0	8.1
1982	299.5	12.4	18.2	6.6
1983	332.8	13.2	18.9	7.5
1984	333.7	13.2	18.6	7.8
Mean	351.8	13.1	18.8	7.4

* From Meterorological division. 1975-1984. Annual meterological summary for Edmonton, from the municipal airport. Metoerology division, Geography department, University of Alberta, Edmonton, Canada.

Appendix 7. Dry matter forage yield (kg/ha) (mean of three replications) diallel I-8x8, 1982A.

	2G169	1G169	1VP9	1V13	2R187	2B29	2B87	2B76	Mean
2G169	0	3074	4169	3222	3510	4090	5485	4749	4043
1G169	3156	0	3222	3663	4255	5589	4860	4728	4210
2B183	1691	2514	0	3160	2827	2259	3107	3374	2705
1VP9	2996	3177	2317	0	4452	3012	2995	4951	3414
2V13	2086	1819	1889	1959	0	2321	3325	3811	2459
2R187	3193	1864	2000	2416	2049	0	2807	2778	2444
2B29	2382	2551	2165	2206	2189	2650	0	3370	2502
2B76	2342	1901	3198	3309	3206	2173	2790	0	2702
Mean	2549	2414	2708	2848	3213	3156	3624	3966	3060

Appendix 8. Dry matter forage yield (kg/ha) (mean of three replications) diallel I-8x8, 1982B.

	2G169	1G169	1VP9	1V13	2R187	2B29	2B87	2B76	Mean
2G169	0	4292	5329	4609	5123	4390	5815	5481	5006
1G169	3584	0	3708	5411	5198	5634	5374	5580	4927
2B183	3148	3572	0	5737	4123	3309	3996	3951	3977
1VP9	4346	4255	3827	0	5593	4119	4486	5593	4603
2V13	3016	3074	2996	2621	0	3078	5074	539	3608
2R187	4259	3136	3160	4885	3041	0	4329	3992	3829
2B29	3255	3432	3337	3621	2996	3358	0	3988	3427
2B76	3798	3321	4481	4749	4490	3189	3531	0	3937
Mean	3629	3583	3834	4519	4366	3868	4658	4854	4164

Appendix 9. Dry matter forage yield (kg/ha) (mean of three replications) diallel I-8x8, 1982T.

	2G169	1G169	1VP9	1V13	2R187	2B29	2B87	2B76	Mean
2G169	0	7366	9498	7831	8634	8481	11300	10230	9049
1G169	6741	0	6930	9074	9453	11222	10235	10309	9137
2B183	4840	6086	0	8897	6951	5578	7103	7325	6681
1VP9	7341	7432	6144	0	10045	7132	7481	10543	8017
2V13	5103	4893	4885	4580	0	5399	8399	9206	6066
2R187	7453	5000	5160	7300	5090	0	7136	6770	6273
2B29	5638	5984	5502	5827	5185	6008	0	7358	5929
2B76	6140	5222	7679	5058	7696	5362	6321	0	6640
Mean	6179	5998	6543	7367	7579	7025	8282	8220	7224

Appendix 10. Dry matter forage yield (kg/ha) (mean of three replications) diallel I-8x8, 1983A.

	2G169	1G169	1VP9	1V13	2R187	2B29	2B87	2B76	Mean
2G169	0	5333	8021	6403	6502	6667	8461	6527	6845
1G169	5218	0	4519	7313	7358	7543	6881	7626	6637
2B183	4947	5473	0	5111	5535	3362	6202	5029	5094
1VP9	7226	6852	6111	0	7185	5589	5708	4860	6219
2V13	4835	4329	4115	4370	0	5704	7580	6510	5349
2R187	6716	4098	5403	5716	4502	0	6465	6589	5641
2B29	5626	4844	5613	4535	4634	5095	0	4066	4916
2B76	6025	5453	5346	7305	6119	5494	5770	0	5930
Mean	6646	519	5590	5822	5976	5636	6724	5887	5829

Appendix 11. Dry matter forage yield (kg/ha) (mean of three replications) diallel I-8x8, 1983B.

	2G169	1G169	1VP9	1V13	2R187	2B29	2B87	2B76	Mean
2G169	0	1609	2539	2852	2434	4029	3300	2823	2797
1G169	2440	0	2584	4185	5313	3403	3337	3831	3585
2B183	2481	2823	0	3893	2996	2523	3568	2123	2915
1VP9	3745	4276	2325	0	3506	3012	2741	2523	3161
2V13	2123	2630	2539	3000	0	3173	3551	4305	3046
2R187	3305	2366	2284	3728	2510	0	3144	3630	2995
2B29	2173	3095	2728	2819	2663	2675	0	3053	2744
2B76	2387	3090	2938	3527	2181	2642	2634	0	2771
Mean	2665	2841	2563	3429	3085	3065	3182	3184	3001

Appendix 12. Dry matter forage yield (kg/ha) (mean of three replications) diallel I-8x8, 1983T.

	2G169	1G169	1VP9	1V13	2R187	2B29	2B87	2B76	Mean
2G169	0	6942	10560	9255	8926	10695	11761	9350	9641
1G169	7659	0	7103	11478	12671	10947	10208	11457	8944
2B183	7428	8296	0	9004	8531	5885	9770	7152	8009
1VP9	10971	11128	8436	0	10691	8601	8449	7383	9380
2V13	6959	6959	6654	7370	0	8877	11132	10815	8395
2R187	1002	6465	7687	9444	7012	0	9609	10218	8637
2B29	7798	7938	8342	7354	7296	7770	0	7119	7659
2B76	8411	8543	8284	10831	8300	8136	8403	0	8701
Mean	8464	8039	8152	9251	8061	8701	9906	9071	8830

Appendix 13. Dry matter forage yield (kg/ha) (mean of three replications) diallel I-8x8, 1984A.

	2G169	1G169	1VP9	1V13	2R187	2B29	2B87	2B76	Mean
2G169	0	3407	2481	3877	4654	3716	4333	3000	3638
1G169	3457	0	2568	4506	5074	3284	4568	2494	3707
2B183	3222	6074	0	3037	2889	2012	3235	1827	3185
1VP9	5938	7185	4926	0	5481	3605	5790	1420	4764
2V13	4840	4519	3284	3407	0	2642	4000	3420	3730
2R187	5704	5630	4012	5185	4407	0	3148	3556	4520
2B29	4123	4790	3840	5000	5667	4148	0	2284	4265
2B76	5630	5173	4654	5716	5605	4333	5420	0	5219
Mean	4114	5254	3681	4390	4825	3391	4213	2572	4129

Appendix 14. Dry matter forage yield (kg/ha) (mean of three replications) diallel I-8x8, 1984B.

	2G169	1G169	1VP9	1V13	2R187	2B29	2B87	2B76	Mean
2G169	0	2198	1605	2469	3420	2049	2358	1802	2272
1G169	1802	0	1741	3469	2741	1951	2593	1198	2214
2B183	2395	3148	0	2074	1741	1025	2086	1309	1968
1VP9	3346	3716	2914	0	3000	2185	2556	1383	2729
2V13	2309	2235	2037	1926	0	2198	2716	2259	2240
2R187	3556	3037	2469	2691	2946	0	2210	2716	2718
2B29	1901	2346	2494	2667	3235	2333	0	1432	2344
2B76	3123	2494	2494	3185	3160	2358	2728	0	2792
Mean	2633	2739	2251	2640	2806	2014	2464	1728	2409

Appendix 15. Dry matter forage yield (kg/ha) (mean of three replications) diallel I-8x8, 1984T.

	2G169	1G169	1VP9	1V13	2R187	2B29	2B87	2B76	Mean
2G169	0	5605	4086	6350	8074	5765	6691	4802	5910
1G169	5259	0	4309	7975	7815	5235	7160	3691	5921
2B183	5617	9222	0	5111	4630	3037	5321	3136	9011
1VP9	9284	10901	7840	0	8481	5790	7346	2802	7492
2V12	7148	6753	5321	5333	0	4840	6716	5679	5970
2R187	9259	8667	6481	7877	6753	0	5358	6272	7238
2B29	6025	7136	6333	7667	8901	6481	0	3716	8010
2B76	8753	7667	7148	8901	8765	6691	8148	0	6608
Mean	7335	7993	5931	7030	7631	5406	6677	42999	6538

Appendix 16. Dry matter forage yield (kg/ha) (mean of three replications) diallel I-8x8, 1982-3-4T.

	2G169	1G169	1VP9	1V13	2R187	2B29	2B87	2B76	Mean
2G169	0	19914	24144	23432	25634	24842	29753	29753	24600
1G169	19659	0	18341	28547	29938	27403	27613	25457	25280
2B183	17885	23605	0	23012	20111	14490	22193	17613	19844
1VP9	27597	29461	22420	0	29218	21523	23276	20728	24889
2V13	19210	18605	16860	17284	0	19915	26247	2500	20432
2R187	26733	20132	19329	24621	18856	0	22103	23259	22148
2B29	19461	21058	20177	20848	21283	20250	0	18193	20197
2B76	23305	21432	23111	27790	24761	20189	22872	0	23351
Mean	21978	22029	20626	23648	24272	21132	24865	22190	22593

Appendix 17. Dry matter forage yield (kg/ha) (mean of six replications) diallel II-9x9, 1983T.

	2G169	1G169	2B183	1VP9	2V13	2R187	2B29	2B76	Beaver	Mean
2G169	0	2807	3300	3284	2938	4123	3078	3152	3276	3245
1G169	2897	0	2790	3383	3111	3449	3770	4008	3909	3415
2B183	4033	3630	0	3613	3695	4148	3374	3539	3432	3683
1VP9	4551	4403	4099	0	3465	4000	3473	3646	4222	3982
2V13	3169	3202	3877	3284	0	3942	3309	3012	3004	3350
2R187	3523	4379	3852	4107	3728	0	3646	4362	3350	3868
2B29	3663	3918	3909	3811	3210	4082	0	3483	3350	3868
2B76	3786	4016	4551	3407	3012	4576	4346	0	3473	3896
Beaver	3984	3267	3185	4354	3012	3523	3819	4321	0	3683
Mean	3701	3703	3696	3655	3272	3980	3602	3689	3559	3651

Appendix 18. Dry matter forage yield (kg/ha) (mean of six replications) diallel II-9x9, 1984A.

	2G169	1G169	2B183	1VP9	2V13	2B187	2B29	2B76	Beaver	Mean
2G169	0	6272	7111	6486	6782	7021	7070	6872	7424	6880
1G169	5259	0	5416	5827	5984	5481	5185	6535	5449	5642
2B183	7309	5366	0	6667	7103	6016	5119	6461	5671	6214
1VP9	5646	5235	7095	0	7037	6486	7053	7053	6889	6562
2V13	5564	5128	6066	6798	0	6461	6881	5893	8058	6356
2B187	6782	6576	7029	6082	5909	0	7333	6938	6930	6697
2B29	5712	5663	5695	6477	5827	7662	0	6288	6453	6222
2B76	7761	5786	6033	5424	5300	7177	6626	0	7078	6398
Beaver	7794	5868	5868	5276	7473	6502	5235	7547	0	6445
Mean	6478	5737	6289	6130	5177	6601	6313	6698	6744	6379

Appendix 19. Dry matter forage yield (kg/ha) (mean of six replications) diallel II-9x9, 1984B.

	2G169	1G169	2B183	1VP9	2V13	2B187	2B29	2B76	Beaver	Mean
2G169	0	3053	2905	3325	3350	3424	3309	3514	3086	3246
1G169	2461	0	2864	2782	2691	2724	2543	3119	2741	2741
2B183	3374	3045	0	3309	3572	3136	2724	3539	2502	3150
1VP9	2938	3045	3062	0	3325	3449	2889	3160	3119	3123
2B187	3638	3111	3358	3078	2708	0	3663	3580	3276	3301
2B29	2963	2551	2716	2667	3053	3819	0	2889	2938	2950
2B76	3671	2436	3580	2938	2700	3671	3144	0	3193	3167
Beaver	3292	3062	3119	2757	3185	3218	2716	3292	0	3080
Mean	3151	2862	3076	2972	3073	3290	2981	3199	2989	3066

Appendix 20. Dry matter forage yield (kg/ha) (mean of six replications) diallel II-9x9, 1984T.

	2G169	1G169	2B183	1VP9	2V13	2B187	2B29	2B76	Beaver	Mean
2G169	0	9325	10016	9811	10132	10444	10379	10387	10510	10125
1G169	7720	0	8280	8609	8675	8206	7728	9654	8189	8382
2B183	10683	8412	0	9975	10675	9152	7844	10000	8173	9364
1VP9	8584	8280	10157	0	10362	9934	9942	10214	10008	9685
2V13	8436	7720	9070	9720	0	9350	9737	8395	11111	9685
2B187	10420	9687	10387	9160	8617	0	10995	10519	10206	9999
2B29	8675	8214	8412	9144	8881	11481	0	9177	9391	9172
2B76	11432	8222	9613	8362	8000	10848	9770	0	10272	9565
Beaver	11086	8930	8988	8032	10658	9720	7951	10840	0	9526
Mean	9629	8599	9365	9989	9500	9892	9293	9899	9733	9446

Appendix 21. Dry matter forage yield (kg/ha) (mean of six replications) diallel II-9x9, 1983-84T.

	2G169	1G169	2B183	1VP9	2V13	2B187	2B29	2B76	Beaver	Mean
2G169	0	12131	13316	13095	13070	14568	13457	13539	13786	13370
1G169	1617	0	11070	11992	11786	11654	11498	13663	12099	11797
2B183	14716	12041	0	13588	14370	13300	11218	13539	11605	13047
1VP9	13136	12684	14255	0	13827	13934	13416	13860	14230	13668
2V13	11605	10922	12947	13004	0	13292	13045	11407	14115	12542
2B187	13942	14066	14239	13267	12346	0	14642	14881	13556	13867
2B29	12337	12132	12321	12955	12091	15564	0	12650	13193	12905
2B76	15218	12239	14165	11770	11012	15424	14115	0	13745	13461
Beaver	15070	12198	12173	13671	13243	13243	11770	15760	0	13209
Mean	13330	12302	13060	12757	12771	13872	12895	13588	13291	13096

Appendix 22. Dry matter forage yield (kg/ha) (mean of nine replications) diallels I and II.

	2G169	1G169	1VP9	1V13	2R187	2B29	2B87	2B76	Mean
2G169	0	32045	37460	36527	38704	39536	43210	37922	39340
1G169	21276	0	29411	40539	41724	39057	39111	39120	37034
2B183	32601	35646	0	36600	34481	27790	33411	31152	33097
1VP9	40733	42144	36675	0	43045	35457	36692	34588	38476
2V13	30815	29527	29807	30288	0	32407	39292	37107	32749
2R187	40675	34198	33567	37888	31202	0	36745	38140	36059
2B29	31798	33190	32498	33803	33474	35814	0	30843	33061
2B76	38523	33671	37276	39560	35773	35613	36987	0	36772
Mean	35060	35217	33382	36458	36914	30594	37921	35553	35699

Appendix 23. Plant height (cm) (mean of six replications) diallel III.

	2G169	1G169	2B183	1VP9	2V13	2B187	2B29	2B76	Beaver	Mean
2G169	0	31	33	29	33	33	31	28	28	31
1G169		0	32	29	33	31	31	31	31	31
2B183			0	31	31	32	31	37	25	32
1VP9				0	29	31	32	29	24	29
2V13					0	32	28	29	30	31
2B187						0	32	29	34	32
2B29							0	32	34	31
2B76								0	28	30
Beaver									0	29

Appendix 24. Leaf area (cm²) (mean of six replications) diallel III.

	2G169	1G169	2B183	1VP9	2V13	2B187	2B29	2B76	Beaver	Mean
2G169	0	283.2	320.6	184.5	308.4	303.7	194.8	181.6	254.8	254.0
1G169		0	278.1	256.6	275.5	248.9	197.9	196.2	205.4	242.7
2B183			0	278.3	311.1	268.0	242.9	256.9	226.9	266.0
1VP9				0	205.9	306.1	190.9	200.9	192.0	226.8
2V13					0	193.3	237.2	277.1	171.4	247.5
2B187						0	216.3	272.3	232.2	255.1
2B29							0	194.0	282.0	219.5
2B76								0	228.7	226.0
Beaver									0	224.2

Appendix 25. Leaf dry weight (g) (mean of six replications) diallel III.

	2G169	1G169	2B183	1VP9	2V13	2B187	2B29	2B76	Beaver	Mean
2G169	0	.6436	.7388	.5836	.7145	.8989	.6782	.4248	.6334	.6695
1G169		0	.6856	.5191	.6945	.6270	.5932	.4038	.5709	.5922
2B183			0	.6291	.4535	.7636	.5837	.4971	.5259	.6097
1VP9				0	.5629	.8360	.6000	.7639	.4651	.6199
2V13					0	.5165	.5443	.6280	.5470	.5827
2B187						0	.4999	.5890	.7207	.6815
2B29							0	.5589	.6545	.5891
2B76								0	.7975	.5829
Beaver									0	.6144

Appendix 26. Stem dry weight (g) (mean of six replications) diallel III.

	2G169	1G169	2B183	1VP9	2V13	2B187	2B29	2B76	Beaver	Mean
2G169	0	.6913	.8815	.6540	.8001	1.0815	.7517	.5059	.7081	.7593
1G169		0	.7955	.5722	.7453	.7308	.7016	.4979	.6291	.6703
2B183			0	.7220	.5658	.8598	.6324	.5839	.6004	.2052
1VP9				0	.6819	.9087	.5455	.8323	.5528	.6837
2V13					0	.6110	.6265	.7170	.6372	.6731
2B187						0	.5949	.6945	.8474	.7912
2B29							0	.5937	.7612	.6509
2B76								0	.8571	.6603
Beaver									0	.6992

Appendix 27. Total weight (g)(mean of six replications) diallel III.

	2G169	1G169	2B183	1VP9	2V13	2B187	2B29	2B76	Beaver	Mean
2G169	0	1.3349	1.6203	1.2376	1.5146	1.9804	1.4299	.9307	1.3414	1.4287
1G169		0	1.4811	1.0913	1.4398	1.3578	1.2948	.9017	1.2000	1.2627
2B183			0	1.3511	1.1008	1.6234	1.2161	1.0810	1.1263	1.3148
1VP9				0	1.2448	1.7447	1.1455	1.5962	1.0170	1.3036
2V13					0	1.1275	1.1708	1.3450	1.1842	1.2558
2B187						0	1.0948	1.2835	1.5681	1.4676
2B29							0	1.1526	1.4157	1.2400
2B76								0	1.6546	1.2432
Beaver									0	1.3135

Appendix 28. Specific leaf weight (mg/cm') (mean of six replications) diallel III.

	2G169	1G169	2B183	1VP9	2V13	2B187	2B29	2B76	Beaver	Mean
2G169	0	2.27	2.30	3.16	2.32	2.96	3.48	2.34	2.49	2.81
1G169		0	2.47	2.02	2.52	2.51	3.00	2.06	2.78	2.58
2B183			0	2.26	1.46	2.85	2.40	1.93	2.32	2.37
1VP9				0	2.73	2.73	3.14	3.80	2.42	2.91
2V13					0	2.67	2.29	2.27	3.19	2.96
2B187						0	2.31	2.16	3.10	2.79
2B29							0	2.88	2.32	2.85
2B76								0	3.49	2.74
Beaver									0	2.89

Appendix 29. Root disease score (1-5)(mean of six replications) diallel III.

	2G169	1G169	2B183	1VP9	2V13	2B187	2B29	2B76	Beaver	Mean
2G169	0	3.0	2.9	2.6	2.2	1.5	1.3	1.9	2.7	2.3
1G169		0	2.8	2.4	2.6	2.2	2.6	2.0	2.3	2.5
2B183			0	3.0	2.3	2.3	1.9	2.1	2.4	2.5
1VP9				0	2.5	1.7	2.8	4.0	2.8	2.2
2V13					0	2.0	1.8	2.0	2.9	2.1
2B187						0	2.1	2.3	1.6	2.0
2B29							0	1.7	2.5	2.1
2B76								0	2.1	2.3
Beaver									0	2.4